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Washington, D.C. 20460

Attn: Gwen Shepherd
TSCA § 4

RE: Sampling Plan, Quality Assurance Project Plan and Analytical Protocol
Company Sanitized Version

Dear Sir or Madam:

Enclosed pursuant to 40 C.F.R. Part 766 are six (6) copies of a company-sanitized Sampling Plan, Quality Assurance Project Plan and Analytical Protocol for testing [*information redacted*], CAS No. [*information redacted*]. In my letter to you dated March 15, 2000, I erroneously stated that the company was abandoning its claim of business confidentiality for the identity of this test substance. **In fact, Albaugh does wish to maintain its claim of confidentiality for the test substance.** Albaugh acknowledges that "health and safety data" regarding the test substance as defined in 40 C.F.R. § 2.306(a)(3) are not eligible for confidential treatment.

I apologize for the confusion, and please do not hesitate to call with any further questions that you may have. A sanitized version of this letter is also enclosed for your use.

Sincerely,

Stuart D. Feldstein

Stuart I. Feldstein
General Counsel

COMPANY SANITIZED

Encls.

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ANALYTICAL PROTOCOL

DETERMINATION OF POLYCHLORONATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN REDACTED

Test Substances:

Redacted

Prepared For:

Albaugh, Inc.
121 N. E. 18th Street
Ankeny, IA 50021
(515) 964-9444

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ANALYTICAL PROTOCOL
DETERMINATION OF POLYCHLORONATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN
REDACTED

APPROVED, BATTELLE:

Mark R. Bauer — 3/15/00
Mark R. Bauer, Ph.D.
Study Director
Battelle
Date

Stephen J. Swanson for Corazon A. Steginsky, Ph.D. — 3/15/00
Corazon A. Steginsky, Ph.D.
Program Management
Battelle
Date

Charles V. Jaworski — 3/15/00
Quality Assurance Unit
Battelle
Date

APPROVED, SPONSOR:

Ron Collins — 3/16/00
Ron Collins
Study Monitor
Albaugh, Inc.
Date

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ANALYTICAL PROTOCOL DETERMINATION OF POLYCHLOROINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN REDACTED

1.0. Principals

1.1 Sponsor:

Albaugh, Inc.
121 N. E. 18th Street
Ankeny, IA 50021
Phone: (816) 238-2844
Fax: (816) 238-3938

Study Monitor -

Ron Collins
Albaugh, Inc.
4900 Packers Avenue
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1.2 Testing Facility:

Battelle
505 King Avenue
Columbus, Ohio 43201-2693
(614) 424-6424

Study Director -

Mark R. Bauer, Ph.D.
Phone: (614) 424-3913
Fax: (614) 424-3742

2.0 Proposed Experimental Start Date July 1, 2000

3.0 Proposed Experimental Termination Date September 1, 2000

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4.0 Data Requirements

U.S. Environmental Protection Agency (EPA) Toxic Substance Control Act (TSCA); Good Laboratory Practice Standards, 40 CFR Part 792; and the Dioxin/Furan Test Rule, 40 CFR Part 766. Under these provisions, this study is defined as a physical and chemical characterization study designed to determine certain physical and chemical characteristics of the test substance redacted (see 792.135(b); (54 FR 34094)).

5.0 Objective

The objective of this study is to generate data of known and acceptable precision and accuracy on the levels of 2,3,7,8-substituted tetra- through hepta- chlorinated dibenzo-p-dioxins and dibenzofurans in redacted imported by Albaugh, Inc. The analysis results will be used to meet regulatory requirements promulgated by the U.S. Environmental Protection Agency; Dioxin/Furan Test Rule, 40 CFR Part 766.

6.0 Test Substance, Reference Substances, and Test System

6.1 Test Substance

Redacted is the test substance for this study. Albaugh, Inc. will maintain a certificate of analysis and other chemical and physical characterization as required by Good Laboratory Practice Standards. Albaugh, Inc. will also maintain the stability, storage conditions, and will maintain the documentation for the synthesis and characterization of the test substances.

Common Name:	redacted
Chemical Name:	redacted
CAS Number:	redacted
Empirical Formula:	redacted
Molecular Weight:	redacted
Structure	redacted

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6.2 Reference Substances

The reference substances listed below will be used for this study. The reference substances will be obtained in pre-mixed solutions from Cambridge Isotope laboratories (CIL) who will provide a certificate of analysis and other chemical and physical characterization as required by Good Laboratory Practice Standards. Cambridge Isotope Laboratories will also supply the stability, storage conditions, and will maintain the documentation for the synthesis and characterization of the reference substances.

Name:	2,3,7,8-tetrachlorodibenzo-p-dioxin
CAS Number:	1746-01-6
Chemical Formula:	$C_{12}H_4Cl_4O_2$

Name:	2,3,7,8-tetrachlorodibenzofuran
CAS Number:	51207-31-9
Chemical Formula:	$C_{12}H_4Cl_4O$

Name:	1,2,3,7,8-pentachlorodibenzo-p-dioxin
CAS Number:	40321-76-4
Chemical Formula:	$C_{12}H_3Cl_5O_2$

Name:	1,2,3,7,8-pentachlorodibenzofuran
CAS Number:	57117-41-6
Chemical Formula:	$C_{12}H_3Cl_5O$

Name:	2,3,4,7,8-pentachlorodibenzofuran
CAS Number:	57117-31-4
Chemical Formula:	$C_{12}H_3Cl_5O$

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Name:	1,2,3,4,7,8-hexachlorodibenzo-p-dioxin
CAS Number:	39227-28-6
Chemical Formula:	$C_{12}H_2Cl_6O_2$

Name:	1,2,3,6,7,8-hexachlorodibenzo-p-dioxin
CAS Number:	57653-85-7
Chemical Formulas:	$C_{12}H_2Cl_6O_2$

Name:	1,2,3,7,8,9-hexachlorodibenzo-p-dioxin
CAS Number:	19408-74-3
Chemical Formulas:	$C_{12}H_2Cl_6O_2$

Name:	1,2,3,4,7,8-hexachlorodibenzofuran
CAS Number:	70648-26-9
Chemical Formula:	$C_{12}H_2Cl_6O$

Name:	1,2,3,6,7,8-hexachlorodibenzofuran
CAS Number:	57117-44-9
Chemical Formula:	$C_{12}H_2Cl_6O$

Name:	1,2,3,7,8,9-hexachlorodibenzofuran
CAS Number:	72918-21-9
Chemical Formula:	$C_{12}H_2Cl_6O$

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Name:	2,3,4,6,7,8-hexachlorodibenzofuran
CAS Number:	60851-34-5
Chemical Formula:	$C_{12}H_2Cl_6O$

Name:	1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin
CAS Number:	35822-46-9
Chemical Formula:	$C_{12}HCl_7O_2$

Name:	1,2,3,4,6,7,8-heptachlorodibenzofuran
CAS Number:	67562-39-4
Chemical Formula:	$C_{12}HCl_7O$

Name:	1,2,3,4,7,8,9-heptachlorodibenzofuran
CAS Number:	55673-89-7
Chemical Formula:	$C_{12}HCl_7O$

Name:	$^{13}C_{12}$ -2,3,7,8-tetrachlorodibenzo-p-dioxin
CAS Number:	76523-40-5
Chemical Formula:	$^{13}C_{12}H_4Cl_4O_2$

Name:	$^{37}Cl_4$ -2,3,7,8-tetrachlorodibenzo-p-dioxin
CAS Number:	85508-50-5
Chemical Formula:	$C_{12}H_4{}^{37}Cl_4O_2$

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Name:	$^{13}C_{12}$-2,3,7,8-tetrachlorodibenzofuran
CAS Number:	89059-46-1
Chemical Formula:	$^{13}C_{12}H_4Cl_4O$
Name:	$^{13}C_{12}$-1,2,3,7,8-pentachlorodibenzo-p-dioxin
CAS Number:	109719-79-1
Chemical Formula:	$^{13}C_{12}H_3Cl_5O_2$
Name:	$^{13}C_{12}$-1,2,3,7,8-pentachlorodibenzofuran
CAS Number:	109719-77-9
Chemical Formula:	$^{13}C_{12}H_3Cl_5O$
Name:	$^{13}C_{12}$-2,3,4,7,8-pentachlorodibenzofuran
CAS Number:	116843-02-8
Chemical Formula:	$^{13}C_{12}H_3Cl_5O$
Name:	$^{13}C_{12}$-1,2,3,4,7,8-hexachlorodibenzo-p-dioxin
CAS Number:	109719-80-4
Chemical Formula:	$^{13}C_{12}H_2Cl_6O_2$
Name:	$^{13}C_{12}$-1,2,3,6,7,8-hexachlorodibenzo-p-dioxin
CAS Number:	109719-81-5
Chemical Formula:	$^{13}C_{12}H_2Cl_6O_2$

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Name:	$^{13}C_{12}$-1,2,3,7,8,9-hexachlorodibenzo-p-dioxin
CAS Number:	109719-82-6
Chemical Formula:	$^{13}C_{12}H_2Cl_6O_2$
Name:	$^{13}C_{12}$-1,2,3,4,7,8-hexachlorodibenzofuran
CAS Number:	114423-98-2
Chemical Formula:	$^{13}C_{12}H_2Cl_6O$
Name:	$^{13}C_{12}$-1,2,3,6,7,8-hexachlorodibenzofuran
CAS Number:	116843-03-9
Chemical Formula:	$^{13}C_{12}H_2Cl_6O$
Name:	$^{13}C_{12}$-1,2,3,7,8,9-hexachlorodibenzofuran
CAS Number:	116843-04-0
Chemical Formula:	$^{13}C_{12}H_2Cl_6O$
Name:	$^{13}C_{12}$-2,3,4,6,7,8-hexachlorodibenzofuran
CAS Number:	116843-05-1
Chemical Formula:	$^{13}C_{12}H_2Cl_6O$
Name:	$^{13}C_{12}$-1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin
CAS Number:	109719-83-7
Chemical Formula:	$^{13}C_{12}HCl_7O$

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Name:	$^{13}C_{12}$-1,2,3,4,6,7,8-hexachlorodibenzofuran
CAS Number:	109719-84-8
Chemical Formula:	$^{13}C_{12}HCl_7O$

Name:	$^{13}C_{12}$-1,2,3,4,7,8,9-hexachlorodibenzofuran
CAS Number:	109719-94-0
Chemical Formula:	$^{13}C_{12}HCl_7O$

6.3 Test System

The test system for this study will be redacted.

6.4 Justification for Selection of the Test System

The test system for this study is the same as the test substance upon which all analyses will be conducted. The test system chosen is the actual substance to which the Dioxin/Furan Test Rule applies

6.5 Identification of the Test System

All test system samples will be labeled with a minimum of a unique Battelle study number and sample identification code. A unique identification code will be used so that no sample numbers are repeated.

7.0 Method

Contamination testing will be conducted using the procedures specified in the appended method entitled "Analytical Method for the Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans by High-Resolution Gas Chromatography/High Resolution Mass Spectrometry in Redacted". The method follows the "Guidelines for the Determination of Halogenated Dibenzo-p-Dioxins and Dibenzofurans in Commercial Products". The method is based on procedures assembled from Dow Chemical's "Procedure for the Analysis of 2,4-Dichlorophenoxyacetic Acid and 2,4-Dichlorophenol for the Presence of Chlorinated Dibenzo-p-dioxins and Chlorinated Dibenzofurans", EPA Method 8290, and EPA Method 1613. The method outlines the analytical procedures, the quality control objectives, and corrective actions that will be applied to the contamination testing.

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A minimum of seven product samples will be collected according to the "Sampling Protocol for Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Redacted". Seven samples will be randomly chosen (or all seven if only seven are collected) and analyzed by the method. One of the seven samples will be analyzed in duplicate to demonstrate that the Test Rule requirements for method sensitivity can be met. A laboratory method blank and a matrix spike sample will also be analyzed concurrently with the seven product samples. The seven test samples, a duplicate, a matrix spike, and a laboratory method blank constitute the sample set.

8.0 Control of Experimental Bias

The experimental design incorporates replicate analyses of a sample, a matrix spike, the use of isotope-labeled internal standards, and averaging of the analytical results to control for experimental bias.

9.0 Statistical Methods

Data will be summarized utilizing averages, standard deviations, relative standard deviations, and relative percent differences. The 95% confidence limit will be used for any statistical evaluations of the data.

10.0 Records to be Maintained

All raw data and written records concerning the study will be included in notebooks established for the study. The study records will be maintained in accordance with Battelle's facility SOP for assembling study files and will include, but not necessarily be limited to, the following:

1. This protocol, and any protocol amendments or deviations;

2. Test substance identification records, characterization records supplied by the sponsor, and receipt and inventory records;

3. A full description of experiments conducted, including a description of the test equipment used;

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4. Study raw data including:

   - sample preparation records
   - mass chromatograms
   - instrument records

5. The final report and any amendments thereto.

11.0 Archive Statement

The study notebook(s), raw data, and other study records will be stored by Battelle, Columbus, Ohio after the approval of the final report. A copy of the final report will be archived with the study records after all approvals have been obtained.

All unused redacted will be returned to Albaugh, Inc. at the end of the study.

12.0 Regulatory Compliance and Quality Assurance

Battelle is committed to performing laboratory studies in compliance with current Good Laboratory Practices established by the U.S. Environmental Protection Agency. A separate independent Quality Assurance Unit (QAU) is responsible for monitoring each study to assure conformance to these regulations. The signature of the study director shall attest to the authenticity of the study. The Battelle QAU will prepare and sign a statement to be included in the final report, which will specify the phases of the study which were inspected, the dates of inspections, and the dates inspection results were reported to the Battelle Study Director and Battelle management.

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APPENDIX 1

ANALYTICAL METHOD

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ANALYTICAL METHOD FOR THE DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS BY HIGH-RESOLUTION GAS CHROMATOGRAPHY / HIGH RESOLUTION MASS SPECTROMETRY IN REDACTED

Prepared by

Mark R. Bauer, Ph.D.

Battelle
505 King Avenue
Columbus, OH 43201-2693

for

Albaugh, Inc.

March, 2000

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STATEMENT OF PURPOSE

In order to satisfy requirements for testing chemical substances that may be contaminated with halogenated dibenzo-p-dioxins and dibenzofurans as defined by Section 4 or U.S. Environmental Protection Agency (EPA) Toxic Substance Control Act (TSCA) 15 USC 2603 and 40 CFR § 766.3 and requirements for reporting under Section 8 of TSCA, 15 USC 2607, Albaugh, Inc. has contracted with Battelle to perform the dioxin/furan contamination study following 40 CFR Part 766 on redacted.

Limits of Quantification (LOQ) established as targets by the regulation for the individual congeners are summarized below:

Chlorinated Dibenzodioxins/Dibenzofurans	LOQ in ppb
2,3,7,8-TCDD	0.1
1,2,3,7,8-PeCDD	0.5
1,2,3,4,7,8-HxCDD	2.5
1,2,3,6,7,8-HxCDD	2.5
1,2,3,7,8,9-HxCDD	2.5
1,2,3,4,6,7,8-HpCDD	100
2,3,7,8-TCDF	1
1,2,3,7,8-PeCDF	5
2,3,4,7,8-PeCDF	5
1,2,3,4,7,8-HxCDF	25
1,2,3,6,7,8-HxCDF	25
1,2,3,7,8,9-HxCDF	25
2,3,4,6,7,8-HxCDF	25
1,2,3,4,6,7,8-HpCDF	1,000
1,2,3,4,7,8,9-HpCDF	1,000

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The purpose of this method is to utilize developed analytical methodologies to detect and quantify polychlorinated dibenzo-p-dioxins (2,3,7,8-substituted tetra- through heptachlorinated homologues) and polychlorinated dibenzofurans (2,3,7,8-substituted tetra- through heptachlorinated homologues) in redacted at part and sub-part per billion levels. The present document contains the method addressing the analytical procedures and the quality assurance requirements for the determination of PCDD/PCDFs in redacted.

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ABBREVIATIONS AND SYMBOLS

A	Integrated ion abundance
C	Concentration
EC	Degree Celsius
^{13}C	Carbon-13 labeled
cm	Centimeter
DB-5	Type of fused-silica capillary column
g	Gram
GC	Gas chromatography or gas chromatograph
GC/MS	Gas chromatography/Mass spectrometry
HpCDD	Heptachlorodibenzodioxin
HpCDF	Heptachlorodibenzofuran
HpCDF	Heptachlorodibenzofuran
HRGC/HRMS	High-resolution gas chromatography/high resolution mass spectrometry
HxCDD	Hexachlorodibenzodioxin
HxCDF	Hexachlorodibenzofuran
IS	Internal standard
L	Liter
LOQ	Limit of quantification
MCL	Method calibration limit
mL	Milliliter
mm	Millimeter
ND	Not detected at or above LOQ
PCDD	Polychlorinated dibenzodioxin
PCDF	Polychlorinated dibenzofuran
PeCDD	Pentachlorodibenzodioxin
PeCDF	Pentachlorodibenzofuran
PFK	Perfluorokerosene
pg	Picogram (10^{-12} g)
ppb	Part per billion
ppm	Part per million
Q	Amount of substance
QA	Quality assurance or quality assessment
QA/QC	Quality assurance/Quality control
RPD	Relative percent difference
RRF	Relative response factor
RRT	Relative retention time
RS	Recovery standard
RT	Retention time

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ABBREVIATIONS AND SYMBOLS (CONTINUED)

SIM	Selected ion monitoring
S/N	Signal-to-noise ratio
SOP	Standard operating procedure
TCDD	Tetrachlorodibenzodioxin
TCDF	Tetrachlorodibenzofuran
V	Volume
W	Weight
µL	Microliter

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PART 1.

ANALYTICAL METHODS

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ANALYTICAL METHODS

1.0 Scope and Application

1.1 This method provides procedures for the detection and quantitative measurement of polychlorinated dibenzo-p-dioxins (2,3,7,8-substituted tetra- through heptachlorinated homologs) and polychlorinated dibenzofurans (2,3,7,8-substituted tetra- through heptachlorinated homologs) in the test substance redacted, a polyhalogenated redacted, at part and sub-part per billion levels. Table 1 lists the 2,3,7,8-TCDD-based method calibration limits, sample size, and other information. The procedure uses stable isotopes of tetra through hepta isomers as internal standards for the quantitation of the native compounds. High-resolution gas chromatography and high-resolution mass spectrometry (HRGC/HRMS) is used to analyze purified sample extracts. Standard reference compounds are used for the identification of the fifteen 2,3,7,8-substituted PCDD/PCDF congeners.

1.2 The procedure is designed to achieve the following limits of quantitation (LOQ):

2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD	0.1 ppb
1,2,3,7,8-pentachlorodibenzo-p-dioxin (1,2,3,7,8-PeCDD)	0.5
1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (1,2,3,4,7,8-HxCDD)	2.5
1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (1,2,3,6,7,8-HxCDD)	2.5
1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (1,2,3,7,8,9-HxCDD)	2.5
1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (1,2,3,4,6,7,8-HpCDD)	100
2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF)	1
1,2,3,7,8-pentachlorodibenzofuran (1,2,3,7,8-PeCDF)	5
2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF)	5
1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF)	25
1,2,3,6,7,8-hexachlorodibenzofuran (1,2,3,6,7,8-HxCDF)	25
1,2,3,7,8,9-hexachlorodibenzofuran (1,2,3,7,8,9-HxCDF)	25
2,3,4,6,7,8-hexachlorodibenzofuran (2,3,4,6,7,8-HxCDF)	25
1,2,3,4,6,7,8-heptachlorodibenzofuran (1,2,3,4,6,7,8-HpCDF)	1,000
1,2,3,4,7,8,9-heptachlorodibenzofuran (1,2,3,4,7,8,9-HpCDF)	1,000

1.3 This method is designed for use by analysts who are experienced with residue analysis and skilled in HRGC/HRMS.

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1.4 Seven product samples will be analyzed by this method. One of the seven samples will be analyzed in duplicate to demonstrate that the Test Rule requirements for method sensitivity can be met. A laboratory method blank and a matrix spike sample will also be analyzed concurrently with the seven product samples. The seven test samples, a duplicate, a matrix spike, and a laboratory method blank constitute the sample set. All ten samples are processed together. The quality assurance samples (matrix spike, duplicate, and blank) must be extracted, cleaned up, and analyzed with the test samples for a valid demonstration of the quality of the test data.

2.0 Summary of the Method

2.1 This procedure uses matrix-specific extraction, analyte-specific cleanup and HRGC/HRMS techniques. A simplified sample preparation flow chart for redacted is shown in Figure 1.

2.2 A 10-g portion of sample is dissolved in acetonitrile and 1N sodium hydroxide. The diluted sample is spiked with a solution containing specified amounts of isotopically ($^{13}C_{12}$) labeled PCDD/PCDF internal standards listed in Table 2 at concentrations at or below the LOQ. Precautions are taken to ensure that a representative aliquot of the original sample is used and that the fortification of the sample is performed on a homogeneous liquid solution.

2.3 The fortified sample is extracted with hexane. The hexane is then concentrated and the sample is subjected to several chromatographic cleanup steps. The extract is applied to an acid/base silica column. The column eluant is applied directly to an alumina column. Both columns are rinsed with hexane, then the alumina column is eluted with hexane:methylene chloride mix. The eluant is concentrated and processed through a carbon column. The carbon column eluant is concentrated, fortified with $^{13}C_{12}$-PCDD recovery standards, transferred to autosamplier vials, and further concentrated to 20 µL for HRGC/HRMS analysis.

2.4 A 1-µL aliquot of the prepared sample solution is injected into a HRGC/HRMS system capable of performing selected ion monitoring at resolving powers of approximately 10,000 (10 percent valley definition).

2.5 The identification of the 2,3,7,8-substituted congeners is based on ratio of the integrated ion abundance of the molecular ion species matching theoretical ratios (Table 8); elution at the exact retention time ±3 seconds of the respective internal standard signal; and simultaneous detection of the two most abundant ions in the molecular ion region (Table 6).

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2.6 Quantification of the individual congeners is accomplished with the aid of a six-point calibration curve for each homologous series, during which the six calibration solutions are analyzed once.

2.7 Generally accepted quality control steps will be taken to ensure the quality of the data. These include the analysis of a duplicate, a laboratory method blank, and a matrix spike along with the seven product samples.

3.0 Definitions

3.1 Polychlorinated Dibenzo-p-Dioxins and Polychlorinated Dibenzofurans

Compounds (Figure 2) that contain from one to seven chlorine atoms. The fifteen 2,3,7,8-substituted PCDDs and PCDFs are shown in Table 3. The number of isomers at different chlorination levels is shown in Table 4.

3.2 Homologous Series

A series of chlorinated dibenzodioxins or dibenzofurans in which each member differs by a chlorine atom from the preceding member.

3.3 Isomer

Defined by the arrangement of chlorine atoms within a homologous series. For example, 2,3,7,8-TCDD is a TCDD isomer.

3.4 Congener

Any compound within a given class of compounds. For example, 2,3,7,8-TCDD and 1,2,3,7,8-PeCDD are congeners.

3.5 Internal Standards

An internal standard is a $^{13}C_{12}$-labeled analogue of a congener. Internal standards are added to all samples including laboratory method blanks and other quality control samples before extraction. Internal standards are used to measure the recovery-corrected concentrations of the indigenous analytes.

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3.6 Native Standards

Unlabeled congeners. Native standards are added to one sample and used to assess recovery of the analytes.

3.7 Recovery Standards

Two recovery standards are used to determine the method overall extraction/fractionation efficiencies (percent recoveries of the internal standards). The recovery standards are added to the purified sample extract before HRGC/HRMS analysis.

3.8 Cleanup Standard

A standard added to the extract prior to cleanup to measure the efficiency of the cleanup process

3.9 Calibration Solutions (Table 5)

Solutions containing known amounts of unlabeled PCDDs and PCDFs, internal standards ($^{13}C_{12}$-labeled PCDDs/PCDFs) and the recovery standards. A set of four solutions is used to determine the instrument response of the unlabeled analytes relative to the internal standards and of the internal standards relative to one of the recovery standards.

3.10 Internal Standard Solution (Table 2)

A solution, containing the internal standards, which is used to spike all samples before extraction and cleanup.

3.11 Recovery Standard Solution (Table 2)

A solution, containing the recovery standards, which is added to the sample extract before HRGC/HRMS analysis.

3.12 Native Fortification Solutions (Table 2)

Solutions, containing native standards, which are used to prepare the matrix spike samples.

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3.13 Laboratory Method Blank

A solution, containing the labeled internal standards, that is carried through all analytical procedure steps except the addition of the sample aliquot to the extraction vessel.

3.14 Relative Response Factor

Response of the mass spectrometer to a known amount of an analyte (or internal standard) relative to a known amount of an internal (or recovery) standard.

3.15 Mass Resolution Check

Standard method used to demonstrate the static resolving power (10 percent valley definition) of the mass spectrometer.

3.16 Matrix

The compound that makes up the bulk of the product sample. In this protocol the matrix is redacted.

3.17 Matrix Spike

A sample that is spiked with both the internal standard solution and with the native fortification solutions prior to the extraction step. The recoveries of the matrix spike compounds are determined and are used to estimate the effect of the sample matrix upon the analytical methodology.

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts or elevated baselines that may cause misinterpretation of the chromatographic data. Analysis of laboratory method blanks detects interferences from these materials.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method is not precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must

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be kept to a minimum by whatever means available. The laboratory is responsible for maintaining a current awareness of OSHA regulations regarding the safe handling of the chemicals specified in this method. Each analyst should be acquainted with potential hazards of the reagents, products, and solvents before commencing laboratory work. Sources of information include material safety data sheets, literature, and other related data. Safety information on sample materials should be requested from the supplier. Disposal of reagents, reactants, and solvents should comply with local, state, and federal laws and regulations.

6.0 Apparatus and Equipment

6.1 High-Resolution Gas Chromatograph/High-Resolution Mass Spectrometer System (HRCG/HRMS)

6.1.1 The GC must be equipped for multiple ramp temperature programming. All required accessories, such as syringes, gases, and capillary columns must be available. The GC injection port must be designed for capillary columns. A heated on-column injection technique is used in this protocol. This method prescribes a 1 µL injection volume for all extracts, blanks, and calibration solutions. Larger or smaller injections are allowed if sensitivity considerations require it; however, it is suggested that the injection volume remain consistent throughout the analyses.

6.1.2 Gas Chromatograph/Mass Spectrometer (GC/MS) Interface -- The GC/MS interface components should withstand 350 C. The interface must be designed so that the separation of PCDD/PCDF isomers achieved in the gas chromatographic column is not appreciably degraded. Cold spots or active surfaces (adsorption sites) in the GC/MS interface can cause peak tailing and peak broadening.

6.1.3 Mass Spectrometer -- The instrument must be able to maintain a static resolving power of approximately 10,000 (10 percent valley). The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with a total cycle time (including the voltage reset and dwell times) of approximately one second or less. At a minimum, the ions listed in Table 6 for each of the SIM descriptors must be monitored. It is important to maintain the same set of ions for both calibration and sample analyses.

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6.1.4 Data System -- A dedicated data system is employed to control the rapid multiple ion monitoring process and to acquire the data. Quantification data (peak areas and heights are used for calculations) and SIM traces (displays of intensities of each ion signal being monitored as a function of time) must be acquired during the analyses and stored. Quantifications may be reported based upon computer-generated peak areas. The data system must be capable of acquiring data at a minimum of 20 ions in a single scan. It is also necessary to have a data system capable of switching to different sets of ions (descriptors) at specific times during an HRGC/HRMS acquisition. The data system should be able to provide hard copies of individual ion chromatograms for selected gas chromatographic time intervals. It should also be able to acquire mass-spectra peak profiles and provide hard copies of peak profiles to demonstrate the required resolving power. The data system should also permit the measurement of noise on the base line.

NOTE: The detector ADC zero setting must allow peak-to-peak measurement of the noise on the base line of every monitored channel.

6.2 GC Column

A 60 m x 0.25 mm I.D. fused silica capillary column with a 0.25 μm DB-5 film (or equivalent) is required. Approximately 5 m of uncoated 0.32 mm fused silica is attached with a zero dead volume fitting to the front of the column as a retention gap. Approximately 6 inches of uncoated 0.53 mm fused silica is attached to the front of the retention gap to allow for on-column injection. The recommended column and GC operating conditions are shown in Table 7.

6.3 Miscellaneous Equipment and Materials

The following items are essential for this method; however, this list does not necessarily constitute an exhaustive compendium of the equipment needed.

6.3.1 Nitrogen evaporation apparatus with variable flow rate.

6.3.2 Balances capable of accurately weighing to 0.01 g and 0.0001 g.

6.3.3 Eppendorf continuously adjustable pipettor with disposable tips (or equivalent).

6.3.4 Laboratory hoods.

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6.3.5 Pipettes, disposable, Pasteur, 150 mm long x 5 mm ID (or equivalent).

6.3.6 GC vials with 300 µL inserts (or equivalent).

6.3.7 10 µL on-column syringes.

6.3.8 Glass bottles, assorted sizes with teflon lined caps.

6.3.9 Disposable concentrator tubes, 114 x 18 mm with caps (or equivalent).

6.3.10 Desiccator.

6.3.11 Glass wool, muffled and stored in a clean glass jar.

6.3.12 Sand bath, play sand.

6.3.13 TurboVap evaporator (Zymark)

NOTES: All glassware that is reused must be scrupulously cleaned and muffled before reuse.

Equivalent equipment and regents may be substituted.

7.0 Reagents and Standard Solutions

7.1 Column Chromatography Reagents

7.1.1 Silica Gel - (ICN Biomedicals, 100-200 mesh 60Δ, or equivalent). Fill a muffled Pyrex tube ¾ full with silica and rinse with 350 mL of methanol followed by 350 mL dichloromethane (DCM), using nitrogen to push the solvent through the silica. While still saturated with DCM, empty the silica in to a Soxhlet extractor with a muffled glass thimble, top with DCM-rinsed muffled glass wool, and extract the silica for at least 3 hours. After extraction pour the silica into a muffled Pyrex tube, place under nitrogen, and heat the tube to 65 °C for 30 minutes to dry the silica. Then activate the silica at 160 °C for 1 hour. After activation allow the silica to cool under nitrogen. The silica may be stored in a muffled glass jar with a Teflon-lined screw cap, inside a dessicator.

7.1.2 Acid Silica Gel – Weigh 35 grams of activated silica gel into a muffled glass jar. Add 15 mL of concentrated sulfuric acid. Cap the jar with a

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Teflon-lined screw cap and shake until the mixture is free of lumps and free flowing. Store in a desiccator.

7.1.3 Basic silica gel – Weigh 35 grams of activated silica gel into a muffled glass jar. Add 17 mL of 1N sodium hydroxide solution, cap the jar with a Teflon-lined screw cap, and shake until the mixture is free of lumps and free flowing. Store in a desiccator.

7.1.4 Alumina – (Sigma, activity grade 1, type WB-2 basic, or equivalent) Fill a muffled Pyrex tube ¾ full with alumina and place in a tube furnace under nitrogen. Heat the tube furnace to 500 °C for at least 12 hours. Cool to room temperature, still under nitrogen. Store in a muffled, glass stoppered flask, in a 130 °C oven. Transfer to a dessicator to cool to room temperature immediately before use. Alumina must be used within 3 days of activation.

7.1.5 CarboPack C Carbon – (Supelco, 60/80 mesh, or equivalent) Used as received. Store in a dessicator.

7.1.6 Celite – (supelco, 545-AW, reagent grade, or equivalent) Used as received. Store in a dessicator.

7.1.7 CarboPack C Carbon/Celite Mixture – Weigh 1.6 g of carbon and 6.4 g of celite into a muffled glass jar. Cap the jar with a Teflon-lined cap and rotate the jar until a uniform mix is obtained. Remove the cap, cover the jar with foil that is punctured with several small holes, and place it in an oven at 130 °C for at least 6 hours. Cool to room temperature in a dessicator before use.

7.2 Desiccating Agent

7.2.1 Sodium Sulfate – (JT Baker, 12-60 mesh, Ultra Resi-Analyzed, or equivalent) Pour approximately 1 kg into a large muffled glass thimble and rinse with 500 mL of DCM. Allow to dry in the thimble for 1 hour. Pour the partially dried sodium sulfate into a shallow tray and cover it with muffled aluminum foil. Heat in an oven for a minimum of 6 hours at 450 °C. Store in a muffled glass container at 130 °C. Prior to use, pour an appropriate amount into a muffled glass jar with a Teflon-lined cap and allow to come to room temperature inside a dessicator.

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7.3 Solvents and Reagents

7.3.1 Acetone, acetonitrile, cyclohexane, dichloromethane, hexane, nonane, and toluene - (available from Burdick & Jackson) High-purity, distilled-in-glass or highest available purity.

7.3.2 Dichloromethane:Hexane 1:1 Solution – Use a muffled graduated cylinder to measure 1000 mL of dichloromethane into a muffled 4-L bottle. Add 1000 mL of hexane and mix well. Cap with a Teflon-lined cap and store at room temperature.

7.3.3 Dichloromethane:Methanol:Toluene 15:4:1 Solution – Use a muffled graduated cylinder to measure 150 mL of dichloromethane into a muffled glass 250 mL reagent bottle. Using muffled graduated cylinders, add 40 mL of methanol and 10 mL of toluene. Mix well, cap with a Teflon-lined screw cap, and store at room temperature.

7.3.4 Dichloromethane:Cyclohexane 1:1 Solution – Use muffled graduated cylinders to measure 100 mL each of dichloromethane and cyclohexane into a muffled glass 250 mL reagent bottle. Mix well, cap with a Teflon-lined screw cap, and store at room temperature.

7.3.5 Sulfuric Acid , concentrated – (EM 95-98%, reagent grade, or equivalent)

7.3.6 Sodium Hydroxide – (JT Baker, Baker Analyzed, pellets, or equivalent)

7.3.7 1N Sodium Hydroxide Solution – Weigh 40 grams of sodium hydroxide and transfer into a 1-L Teflon bottle. Slowly add 1 L of water (bottled reagent grade) and mix until the pellets are completely dissolved. Store in the Teflon bottle with a Teflon cap at room temperature.

7.4 Standards

7.4.1 Native Standards -- (available from Cambridge Isotope Laboratories (CIL) as certified solutions).

7.4.2 $^{13}C_{12}$-Labeled Standards – Isotopically labeled 2,3,7,8-substituted chlorinated dibenzo-p-dioxins and dibenzofurans (available from CIL as certified solutions).

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7.4.3 Mass Spectrometer Calibration Standard - Perfluorokerosene (PFK) high boiling, used as supplied (available from PCR).

7.5 Standard Solutions

7.5.1 Calibration Solutions - Six standard solutions (available from CIL) with known concentrations are used to generate a six point calibration curve for the instrument response. The concentrations of the six solutions are those specified by Method 1613 with one additional calibration standard at concentrations equivalent to ½ the level of CS1. The concentrations of the calibration solutions are listed in Table 5. The solutions are used as received. Store the calibration solutions in amber vials at room temperature.

7.5.2 Continuing Calibration Solution – A solution obtained from CIL (EDF-4141) composed of CS3, Window Defining Mix, and Column Performing Mix. The solution is used as received and stored in amber vials at room temperature.

7.5.3 Internal Standard (IS) Spiking Solution - This solution contains the $^{13}C_{12}$-labeled internal standards at the nominal concentrations listed in Table 2. Dilute IS stock solution (CIL EDF-8999) 1:100 with acetone. Using a calibrated pipette, add 120 µL of IS stock solution to a muffled amber vial and dilute to 12 mL with acetone. It is suggested that the acetone be by weight. Cap the vial with a Teflon-lined screw cap, seal with Teflon tape, and store in a freezer.

7.5.4 Matrix Spike Fortification Solution (a.k.a. Precision and Recovery (PAR) Solution) - This solution contains unlabeled (native) PCDD/PCDF standards at the concentrations given in Table 2. Dilute PAR stock solution (CIL EDF-7999) 1:40 with acetone. Using a calibrated pipette add 50 µL of stock solution to an amber vial with a Teflon-lined screw cap. Add 1950 µL of acetone and mix well. Cap the vial, seal with Teflon tape, and store in the freezer. Prior to use, bring the solution to room temperature.

7.5.5 Cleanup Standard (CS) Solution – This solution contains a labeled standard added to the extract before it is processed through the cleanup columns to monitor the efficiency of the cleanup process. Prepare a CS stock standard by diluting 37Cl4-2,3,7,8-TCDD stock solution (CIL ED-907) 1:50 with nonane. Prepare the CS spiking solution by diluting the stock solution 1:5000 with actone. Using a calibrated pipette, add 3 µL

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of stock solution to an amber vial with a Teflon-lined screw cap. Add 14,997 µL of acetone and mix well. It is suggested that the acetone be measured by weight. Cap the vial, seal with Teflon tape, and store in the freezer. Prior to use, bring the solution to room temperature.

7.5.6 Recovery Standard (RS) Solution - This solution contains the $^{13}C_{12}$-labeled recovery standards at the nominal concentration listed in Table 2. Dilute and combine stock solutions of $^{13}C_{12}$1,2,3,4-TCDD and $^{13}C_{12}$ 1,2,3,7,8,9-HxCDD (CIL ED-911 and ED-996) 1:250 with nonane. Using calibrated pipettors, add 20 µL of each stock solution to a muffled amber vial and then add 4960 µL of nonane. Cap with a Teflon-lines screw cap and store in a freezer.

8.0 Sample Tracking and Preservation

8.1 The sample collection, shipping, handling, and chain-of-custody procedures are not described in this document. Once samples are received at the laboratory, sample tracking procedures will follow standard practices or any applicable SOPs. Each sample will be assigned a unique sample number which will be used to identify the sample in all project documentation and raw data.

8.2 All samples must be protected from light and stored at room temperature.

9.0 Extraction and Cleanup Procedures

9.1 Internal Standard Addition

9.1.1 Weigh out a 10.0-g portion of each of the seven samples into a tared 500-mL round bottom flask. If the sample is solidified, melt the sample using a vented oven set at 60 °C. Prepare a second 10.0-g portion of each of two samples, one to be used for a duplicate analysis and one to be used for a matrix spike.

9.1.2 Dissolve the samples by adding 100 mL of acetonitrile and 100 mL of 1N sodium hydroxide. Sonicate the sample if necessary until solids are no longer observed.

9.1.3 Prepare another flask containing only 100 mL of acetonitrile and 100 mL of 1N sodium hydroxide (i.e. no sample matrix). This sample will be used as the method blank.

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9.1.4 To each sample add 1 mL of the internal standard solution, representing a concentration of internal standard at the LOQ for 2,3,7,8-TCDD.

9.1.5 Add 1 mL of the native fortification solution to the sample designated as the matrix spike, representing native 2,3,7,8-TCDD concentrations at the test rule LOQ and concentrations of the other analytes below LOQ.

9.2 Extraction/Cleanup Procedures

9.2.1 Transfer the sample to a 1-L separatory funnel. Add 100 mL of hexane and shake for 2 minutes. Allow the layers to separate. Drain the bottom water/acetonitrile layer into a second separatory funnel. Transfer the hexane layer into a TurboVap tube. Add 100 mL of hexane to the second separatory funnel, shake for 2 minutes, and allow the layers to separate. Drain the bottom layer back into the original separatory funnel. Add the hexane layer to the TurboVap tube. Extract the aqueous solution a third time with 50 mL of hexane. Discard the bottom layer and add the hexane to the TurboVap tube.

9.2.2 Add 0.5 mL of cleanup standard solution to the TurboVap tube.

9.2.3 Evaporate the hexane extract to approximately 4 mL using a TurboVap.

9.2.4 Prepare acid/base silica columns by cutting the tops off muffled 25-mL disposable pipettes, plugging the bottom with muffled glass wool, adding 3 mL of silica, 4 mL of basic silica, 2 mL of silica, 15 mL of acid silica, and approximately ½ inch of sodium sulfate.

9.2.5 Prepare alumina columns by cutting the tops off muffled 25-mL disposable pipettes, plugging the bottom with muffled glass wool, adding 6 g of alumina, and approximately ½ inch of sodium sulfate.

9.2.6 Rinse the alumina column with 20 mL of hexane. Rinse the silica column with 20 mL of hexane. As the last of the rinse approaches the top of the packing, stack the silica column on top of the alumina column such that the eluate from the silica column drops directly into the alumina column.

9.2.7 Transfer the concentrated hexane sample extract from the TurboVap tube to the silica column using muffled pasture pipettes. Rinse the TurboVap tube 3 times with 4 mL of hexane, adding each rinse to the top of the silica column.

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9.2.8 Elute the stacked columns with 100 mL of hexane, collecting the eluate in a muffled 125-mL muffled glass jar. After the last of the hexane passes through the stacked columns, remove the silica column. Elute the alumina column with 40 mL of dichloromethane:hexane 1:1 solution, collecting the eluate in a fresh muffled TurboVap tube.

9.2.9 Concentrate the extracts to near dryness in a TurboVap with a 46°C water bath. Reconstitute the extract with 1 mL of hexane

9.2.10 Prepare carbon/celite columns by cutting the top and bottom off muffled 10-mL pipettes, plugging one end with glass wool, adding, 0.55 g of CarboPack C carbon/celite mixture, and plugging the top with additional glass wool. Rinse the columns with 5 mL of toluene, 2 mL of dichloromethane:methanol:toluene 15:4:1, 1 mL of dichloromethane:cylcohexane 1:1, and 5 mL of hexane.

9.2.11 Transfer the concentrated hexane extract to the top of the carbon column using muffled pasture pipettes. Rinse the TurboVap tube with 2 x 1-mL, 1 x 2-mL, and 2 x 3-mL hexane, applying each rinse to the top of the column.

9.2.12 Rinse the column with 2 mL of dichloromethane:cylcohexane 1:1 and then 2 mL of dichloromethane:methanol:toluene 15:4:1, discarding the eluant.

9.2.13 Invert the column and elute with 30 mL of toluene, collecting the eluant in a 125-mL round bottom flask.

9.2.14 Concentrate the samples to near dryness using a rotovap with the water bath set to approximately 48°C.

9.2.15 Rinse muffled concentrator tubes with dichloromethane by vortexing for approximately 30 seconds. Discard the rinse. Allow the tubes to dry. Add 20 µL of nonane to the concentrator tube and mark the meniscus with a permanent marker. Add an additional 200 µL of hexane to the tubes and mark the meniscus. Leave the solvents in the tubes.

9.2.16 Transfer the concentrated extracts to the concentrator tubes using muffled pasture pipettes. Rinse the round bottom flasks with 3 x 1-mL of hexane, adding the rinsate to the concentrator tubes.

9.2.17 Concentrate the extracts to the hexane meniscus under a nitrogen stream in a sand bath with the temperature set to approximately 45°C. Rinse the

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round flask with 1 mL of dichloromethane and add the rinsate to the concentrator tube. Concentrate the extract back to the hexane meniscus. Rinse the round flask with 0.5 mL of dichloromethane and add the rinsate to the concentrator tube. Concentrate the extract back to the hexane meniscus.

9.2.18 Add 5 µL of recovery standard solution and vortex to mix.

9.2.19 Transfer the extracts to GC autosampler vials (containing small volume inserts) using a pasture pipette and concentrate the extracts to dryness under a nitrogen stream.

9.2.20 Reconstitute the extracts with 20 µL of nonane.

10.0 Instrumental Analysis Procedures

Analyze the prepared sample extracts by HRGC/MRMS using the procedures below. The laboratory may use the recommended GC column described in Section 6.2, Part 1 or an equivalent. It must be documented that all applicable system performance criteria specified in Section 10.1, Part 1 were met before analysis of any sample is performed. Table 7 provides recommended GC/MS conditions that can be used to satisfy the required criteria. The analysis sequence includes checking the response factors and mass spectrometer resolving power at the beginning and the end of each 12-hour period during which samples are analyzed. The analysis of the laboratory method blank (or a solvent blank) is required between a calibration analysis and any subsequent sample analysis.

10.1 Mass Spectrometer Performance

10.1.1 The mass spectrometer is operated in the electron ionization mode. A static resolving power of approximately 10,000 (10 percent valley definition) will be demonstrated at appropriate masses at the beginning and end of every 12-hour period of operation before any calibration or sample analysis is performed.

10.1.2 Chromatography time for PCDD/PCDFs exceeds the long-term mass stability of the mass spectrometer. Therefore, a mass-drift correction is mandatory. Table 6 offers some suggestions for the lock-mass ions. However, an acceptable lock-mass ion at any mass between the lightest and heaviest ion in each descriptor can be used to monitor and correct mass drifts. The level of the reference compound metered into the ion chamber during HRGC/HRMS analyses should be adjusted so that the amplitude of the most intense selected lock-mass ion signal (regardless

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of the descriptor number) does not exceed the full-scale deflection for a given set of detector parameters. Under these conditions, sensitivity changes that might occur during the analysis can be more effectively monitored.

10.1.3 Using a PFK molecular leak, tune the instrument to meet a resolution of approximately 10,000 (10 percent valley). Document tuning parameters.

10.1.4 Document the instrument resolving power by recording at least one peak profile with in the mass range of each group listed in Table 6. The format of the peak profile representation must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale.

10.2 Calibration

10.2.1 Initial Calibration

Initial calibration is required before any samples are analyzed for PCDD/PCDFs. Initial calibration may also be required if routine calibration does not meet the required criteria.

All six calibration solutions listed in Table 5 are used for the initial calibration.

Analyze a 1-µL portion of each of the six calibration solutions.

10.2.2 Initial Calibration Calculations

Calculate the relative response factors for unlabeled native target analytes ($RRF_{(n)}$) present in the calibration solution relative to their appropriate internal standards and for the $^{13}C_{12}$-labeled internal standards ($RRF_{(s)}$) relative to the recovery standard according to the following formula:

$$RRF_{(n)} = \frac{A_n \times Q_{is}}{A_{is} \times Q_n}$$

$$RRF_{(s)} = \frac{A_{is} \times Q_{rs}}{A_{rs} \times Q_{is}}$$

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where:

A_n = Sum of the integrated ion abundances of the quantification ions for native PCDF/PCDD,

A_{is} = Sum of the integrated ion abundance of the quantification ions for the labeled internal standards,

A_{rs} = Sum of the integrated ion abundances of the quantification ions for the labeled recovery standard,

Q_{is} = Quantity of the internal standard injected (pg),

Q_{rs} = Quantity of the recovery standard injected (pg), and

Q_n = Quantity of the native PCDD/PCDF analyte injected (pg).

Calculate the mean relative response factors ($\overline{RRF}_{(n)}$ and $\overline{RRF}_{(s)}$) and their respective relative standard deviations (%RSD) for the six calibration solutions using the following formula:

$$\overline{RRF}_{(n)} = \frac{1}{6}\sum_{j=1}^{6} RRF_{(n)j}$$

$$\overline{RRF}_{(s)} = \frac{1}{6}\sum_{j=1}^{6} RRF_{(s)j}$$

where:

n = Specific 2,3,7,8-substituted PCDD/PCDF congener

j = Injection number or calibration solution number.

The standard deviation (sample standard deviation) is calculated according to the formula:

$$s = \sqrt{\frac{\sum_{i=1}^{N} (x_i - \overline{x})^2}{(N - 1)}}$$

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where:

x_i = sample isomer concentration

$\overline{x}$ = mean isomer concentration

N = number of samples

The percent relative standard deviation (%RSD) is calculated according to the formula:

$$\% \text{ RSD} = \frac{s}{\overline{x}} \times 100$$

10.2.3. Criteria for Acceptable Initial Calibration

The criteria listed below for acceptable calibration must be met before the analysis is performed.

For each ion chromatogram signal corresponding to the elution of a target analyte or a labeled standard, the signal-to-noise (S/N) ratio must be better or equal to 10:1.

The ratio of integrated ion current for the ions belonging to native PCDD/PCDF analytes and the $^{13}C_{12}$-labeled internal and recovery standards must be within the control limits stipulated in Table 8.

The percent relative standard deviations for the mean response factors, $RRF_{(n)}$ and $RRF_{(s)}$, from each of the determinations must be less 20% for native analytes and 30% for internal standards.

NOTE: If the criteria for acceptable initial calibration listed in Section 10.2.3, Part 1 are met, the analyte specific RRFs can then be considered independent of the analyte quantity for the calculations until the routine calibration criteria (Section 10.2.4, part 1) are no longer met. At such time, new mean RRFs will be calculated from a new set of injections of the calibration solutions.

10.2.4 Continuing Calibration

Continuing calibrations must be performed at the beginning and end of each set of sample analyses after successful mass resolution check.

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Inject 1µL of the continuing calibration solution using the same HRGC/HRMS conditions as used for initial calibration.

10.2.5 Continuing Calibration Calculations

Calculate the relative response factors for unlabeled native target analytes ($RRF_{(n)}$) present in the calibration solution relative to their appropriate internal standards and for $^{13}C_{12}$-labeled internal standards ($RRF_{(s)}$) relative to their recovery standard according to the formula in Section 10.2.2, Part 1.

Calculate the relative deviation from the mean (%RDM) for each RRF according to the following formula:

$$\% \text{ RDM} = \frac{| \text{RRF} - \overline{\text{RRF}} |}{\overline{\text{RRF}}} \times 100$$

where *RRF* is the calculated value from the routine calibration and $\overline{RRF}$ is the mean value established during the initial calibration.

10.2.6 Criteria for Acceptable Continuing Calibration

The following criteria must be met before further analysis is performed. If these criteria are not met, corrective action must be taken.

The relative deviation from the mean for measured RRFs obtained during the routine calibration runs must be less than 20% for native analytes and less than 30% for the internal standards.

The ratio of integrated ion current for the ions belonging to native PCDD/PCDF analytes and the $^{13}C_{12}$-labeled internal and recovery standards must be within the control limits stipulated in Table 8.

For each ion chromatogram signal corresponding to the elution of a target analyte or a labeled standard, the signal-to-noise (S/N) ratio must be better or equal to 10:1.

The valley height between the 2,3,7,8-TCDD chromatographic peak and other tetra-dioxin peaks at the same mass must be less than 25%.

If any of the above criteria are not met, the laboratory will attempt to identify the cause of the problem and, if appropriate, repeat the analysis

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of the calibration solution. If re-injection still present results in which any of the criteria are not satisfied, the entire initial calibration process must be repeated.

10.3 Sample Extract Analyses

Establish the GC/MS operating conditions listed in Table 7 and as described in Section 8. Remove the sample from storage. Inject a 1-µL aliquot of the sample into the gas chromatograph. Acquire SIM data. The same data acquisition and mass spectrometer operating conditions must be used for calibration and sample analyses.

10.3.1 Identification Criteria

For a GC peak to be identified as a 2,3,7,8-substituted PCDD/PCDF congener, it must meet all of the following criteria:

The retention times of the sample components (i.e., the two ions used for quantification purposes listed in Table 6) must be within ±3 s of the retention time of the peak for the labeled internal standard at m/z corresponding to the first characteristic ion (of the set of two; Table 6).

The ratios of the integrated ion currents for the two ions used for quantitative and qualitative purposes must be within the lower and upper control limits stipulated in Table 8.

All ion current intensities must be 10 times noise level for positive identification of a PCDD/PCDF compound or a group of coeluting isomers.

10.3.2 Sample Extract Calculations

For gas chromatographic peaks that have met the criteria outlined above, calculate the concentration of 2,3,7,8-substituted PCDD/PCDF compounds using the formula:

$$\text{Concentration (pg / g)} = \frac{A_n \times Q_{is}}{A_{is} \times W \times \overline{RRF}_{(n)}}$$

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where:

A_n = Sum of the integrated ion abundances of the quantification ions for native PCDD/PCDF,

A_{is} = Sum of the integrated ion abundances of the quantification ions for labeled internal standard,

Q_{is} = Quantity of internal standard added to the sample (pg),

W = Sample weight (g)

$\overline{RRF_{(n)}}$ = Mean relative response factor for the analyte calculated from initial calibration data.

NOTE: Because $^{13}C^{12}$-1,2,3,7,8,9-HxCDD is used as a recovery standard, it cannot be used to quantitate native 1,2,3,7,8,9-HxCDD. Therefore, 1,2,3,7,8,9,-HxCDD is quantitated using the averaged response factor of the other two HxCDDs.

Calculate the percent recovery of the internal standards using the formula:

$$\text{Percent Recovery} = \frac{A_{is} \times Q_{rs}}{Q_{is} \times A_{rs} \times \overline{RRF_{(s)}}} \times 100$$

where:

A_{is} = Sum of the integrated ion abundances of the quantification ions for labeled internal standard,

A_{rs} = Sum of the integrated ion abundances of the quantification ions for the labeled recovery standard.

Q_{rs} = Quantity of recovery standard added to the sample before analysis (pg),

Q_{is} = Quantity of internal standard added to the sample before preparation (pg),

$\overline{RRF_{(s)}}$ = Mean relative response factor for the labeled internal standard calculated from initial calibration data.

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The standard deviation (sample standard deviation) is calculated according to the formula:

$$s = \sqrt{\frac{\sum_{i=1}^{N} (x_i - \overline{x})^2}{(N - 1)}}$$

where:

x_i = sample isomer concentration

$\overline{x}$ = mean isomer concentration

N = number of samples

The percent relative standard deviation (%RSD) is calculated according to the formula:

$$\% \text{ RSD} = \frac{s}{\overline{x}} \times 100$$

The relative percent difference (%RPD) is calculated as follows:

$$\% \text{ RSD} = \frac{|X_1 - X_2|}{\frac{(X_1 + X_2)}{2}} \times 100$$

where X_1 and X_2 represent the recoveries of the labeled internal standards in the regular sample (1) and the duplicate sample (2).

10.4 High Concentration or High Intensity Peaks

If the SIM area for any quantitation m/z exceeds the instrument detector response (as indicated by a flat top GC peak), the sample must be diluted and reanalyzed. Dilute the extract with nonane by a factor that insures that the appropriate internal standard will still have an acceptable response and reanalyze the diluted extract.

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If the calculated concentration of any analyte exceeds the calibration range established during the initial calibration, the sample extract must be diluted and reanalyzed. Dilute the extract with internal standard solution and reanalyze the diluted extract.

11.0 Reporting

The results of the analyses carried out under this protocol will be reported to the U.S. Environmental Protection Agency as directed in 40 CFR Parts 707 and 766, June 5, 1987. The report will include the following items:

- Cover letter
- Title page
- GLP compliance statement
- Summary section
- Sampling section
- Description of sample preparation
- Description of instrumental analysis
- Summary of initial and continuing calibration results
- Results for chemical product samples
- Results for quality control analysis
- Raw data and documentation

11.1 Specific documentation to be included for the above items have been identified by the U.S. Environmental Protection Agency (Steel, D.H., and T. Dux, "Guidelines for Reporting Test Results for HDD and HDF Determinations in Commercial Products (40 CFR Parts 707 and 766)", U.S. Environmental Protection Agency, Final Report, June 22, 1990).

11.2 The summary of initial and continuing calibration results should be presented in tables. Quality control information should be calculated and included in this section. This includes average response factors, percent relative standard

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deviation, and relative percent difference. Data for any analyte that does not meet the required criteria should be clearly identified in the table. The use of instrument calibration that does not meet criteria must be justified in terms of overall data quality and impact on the sample results.

11.3 The section presenting the results for the chemical product samples should be presented in a table. Results which do not meet data quality objectives should be flagged and discussed in the text. Internal standard recovery statistics such as average recovery for a specific analyte, standard deviation, and percent relative standard deviation should be calculated and presented.

11.4 Copies of all raw data and documentation should be included in the form of one or more appendices to the report.

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Figure 1. Sample Extraction and Cleanup Procedure

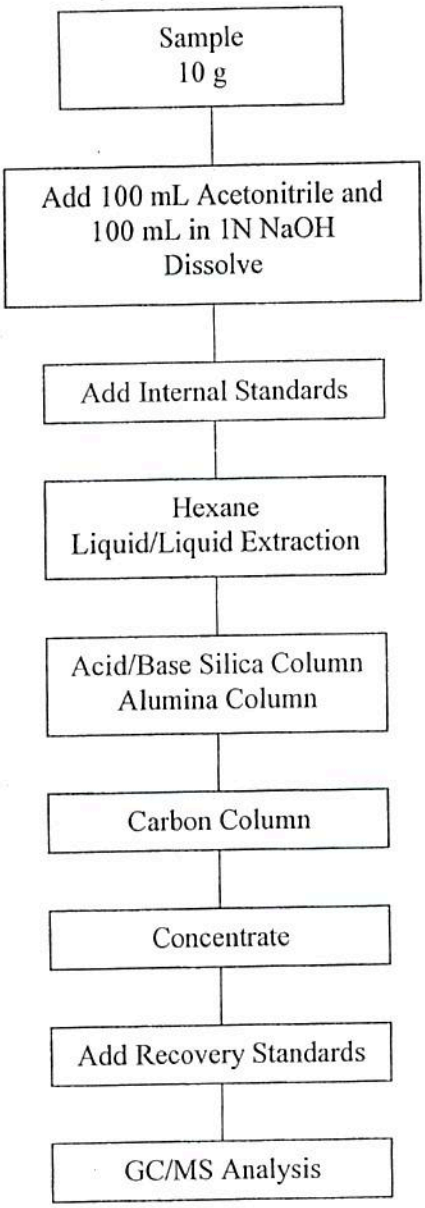

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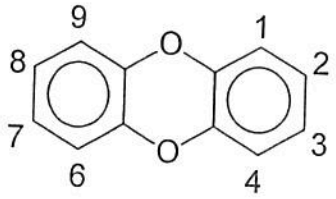
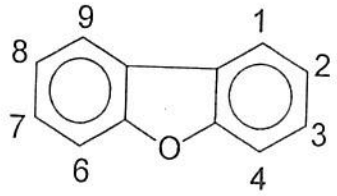
Figure 2. Structure of the PCDDs and PCDFs



Dibenzo-p-dioxin



Dibenzofuran

CHLORINATED DIBENZO-P-DIOXINS		CHLORINATED DIBENZOFURANS	
No. Cl	Structure	No. Cl	Structure
4	2,3,7,8	4	2,3,7,8
5	1,2,3,7,8	5	1,2,3,7,8
6	1,2,3,6,7,8	5	2,3,4,7,8
6	1,2,3,4,7,8	6	1,2,3,6,7,8
6	1,2,3,7,8,9	6	1,2,3,7,8,9
7	1,2,3,4,6,7,8	6	1,2,3,4,7,8
		6	2,3,4,6,7,8
		7	1,2,3,4,6,7,8
		7	1,2,3,4,7,8,9

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PART 2.

QUALITY ASSURANCE REQUIREMENTS

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QUALITY ASSURANCE REQUIREMENTS

1.0 Summary of QA/QC Analyses and Requirements

- Initial Calibration (see Section 10.2.3, Part 1 for criteria)
- Continuing Calibration (beginning and end of each analysis sequence; Section 10.2.4 and 10.2.6, Part 1)
- Instrument Performance Check (Section 10.1, Part 1)
- Laboratory Method Blank (minimum of one per batch of 10 samples or less)
- Internal Standards Percent Recoveries: 50-150%
- Method Sensitivity and Reproducibility: duplicate analyses performed on a product sample fortified with the labeled PCDD/PCDF standards at or below the EPA Test Rule LOQs: %RPD 20% (one set of duplicates per batch of 10 samples or less).
- Matrix Spike: 50 - 150% Accuracy (one matrix spike per batch of 10 samples or less).
- S/N of 10:1 for the Internal Standards
- Quality Control Selected Ion Current Profiles (see Section 3.1.2.4, Part 2)

2.0 Quality Control Procedures

2.1 Performance Check Solutions

2.1.1 A mass resolution check is performed to demonstrate adequate mass resolution using an appropriate reference compound (PFK). These procedures are described in Section 10.1 of Part 1. If the required criteria are not met, corrective action, such as instrument retuning and checking instrument integrity, must be taken before any samples are analyzed.

2.1.2 At the beginning of each 12-hour period during which samples are to be analyzed, an aliquot of a calibration solution is analyzed to demonstrate

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adequate sensitivity, response factor reproducibility, mass range calibration, and chromatographic performance.

2.1.3 At the end of each period during which samples were analyzed, a mass resolution check is performed to demonstrate that the instrument resolution has not changed during the analysis period. A continuing calibration is also performed to demonstrate that the instrument calibration, response, and chromatographic performance have not changed during the analysis period.

2.2 Quality Control Selected Ion Current Profiles

2.2.1 Under high-resolution mass spectrometric conditions, the use of a reference compound such as perfluorokerosene (PFK) is used. The function of the reference compounds is to provide ions that can be used as lock-mass ions to compensate for mass-drift occurring during the analysis.

2.2.2 The ion chromatograms for the lock mass ions are used to demonstrate that the mass calibration has not drifted during the analysis, that the reference compound was not depleted during the analysis, and that abundant unknown compounds are not eluting simultaneously with the target analytes. The Quality Control ion profiles should show a level response during an injection. If acceptable chromatograms are not obtained, the cause of the problem should be investigated and any necessary corrective action taken before any additional samples are analyzed.

2.3 Method Blank

A laboratory method blank sample extract is used to demonstrate that contamination is not introduced from the reagents and laboratory equipment used for the analyses.

2.3.1 One laboratory method blank is required per batch of 10 samples or less. To that effect, perform all steps detailed in the analytical procedure (Part 1) using all reagents, standards, equipment, apparatus, glassware and solvents that would be used for a sample analysis, but omit addition of the product sample.

An acceptable method blank exhibits no positive response (i.e., no response for specific analytes that exceeds the target analytical method

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LOQ). A specific analyte is any 2,3,7,8-substituted PCDD/PCDF congener.

2.3.2 If an acceptable method blank is not obtained, check solvents, reagents, fortification solutions, apparatus and glassware to locate and eliminate the source of contamination before any further samples are extracted and analyzed.

2.4 Identification Criteria

2.4.1 If any of the identification criteria appearing in Section 10.4.1, Part 1, are not met for an homologous series, the sample is reported not to contain 2,3,7,8-substituted PCDD/PCDF isomers for that homologous series at the target limit of quantification.

2.4.2 If the initial identification criteria in Sections 10.4.1, Part 1 are met, but the isotope criteria is not met, the sample is presumed to contain interfering contaminants. Further fractionation to remove the interference may be performed at the sponsors request.

3.0 Data Quality Objectives and Corrective Actions

3.1 Duplicate Samples

In order to demonstrate that the test rule method sensitivity requirements can be met, one of the seven product samples is analyzed in duplicate. Internal standard fortification levels in the duplicate samples must be equal to or below the target LOQs.

The recoveries of the labeled internal standards from each analysis should be in the range of 50 - 150%. The recoveries of labeled 2,3,7,8-substituted PCDD/PCDF standards in the laboratory duplicates must agree within 20 percent (relative percent difference must not exceed 20). If the relative percent difference between recoveries is the two duplicates is greater than 20 for any one of the labeled 2,3,7,8-substituted PCDD/PCDFs, the laboratory will attempt to identify the cause of the problem and repeat the analysis of the duplicates. The re-analysis of the duplicates is performed by re-injecting the cleaned up extracts in the GC/MS system. If the precision and recovery requirements for the duplicates cannot be met upon re-analysis (re-injection) of the duplicates, the entire sample set, including quality assurance samples, should be reprocessed and analyzed.

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NOTE: Re-analysis and/or re-extraction should only be undertaken with permission of the sponsor.

3.2 Matrix Spike

One matrix spike sample is required per batch of 10 samples or less. An appropriate volume of the matrix spike fortification solution is added to an aliquot of one of the samples to give PCDD/PCDF concentrations at the test rule LOQ or below.

The results obtained from the matrix spike sample (concentrations of 2,3,7,8-substituted PCDD/PCDFs) should agree within 50 percent of the expected value. Results are to be corrected for indigenous analytes found in the product sample used for the matrix spike.

If the matrix spike sample presents accuracies falling outside the QC limits, the laboratory will attempt to identify the cause of the problem and, if appropriate, repeat the analysis of the matrix spike sample. Re-analysis of the matrix spike sample is performed by re-injection of the cleaned up extract on the GC/MS system. The entire sample set should be reprocessed and re-analyzed if the matrix spike sample still presents accuracies falling outside the QC limits

3.3 Laboratory Method Blank

The laboratory method blank sample is fortified, extracted and fractionated along with the test samples at a frequency of one laboratory method blank per batch of 10 (or less).

A valid laboratory method blank should present recoveries of the internal standards between 50% and 150%. If interferences are encountered that cause recoveries of the internal standards in the blank to be greater than 150%, the cause should be identified and the appropriate reagents or glassware should be replaced or cleaned up, or the necessary instrumental adjustments should be made. The analysis of the sample set then should be repeated. In the event that recoveries of the internal standards in the blank are less than 50%, the cause should be identified, appropriate procedural changes performed, and the analysis of the sample set repeated.

The laboratory method blank should not show a positive response for specific analytes (i.e., the 2,3,7,8-substituted PCDD/PCDF congeners) that exceeds the Test Rule LOQ.

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If the method blank that was extracted along with a batch of samples is contaminated by PCDD/PCDFs, all positive samples from that batch will be rerun if they are positive for the same analyte(s) for which the blank was positive.

If the method blank that was extracted along with the batch of samples is contaminated by 2,3,7,8-substituted PCDD/PCDFs but the specific analyte concentrations do not exceed 5% of the level found in the product sample(s), the sample set will not have to be re-extracted or analyzed.

If the method blank that was extracted along with the batch of samples is contaminated by 2,3,7,8-substituted PCDD/PCDFs, only those samples showing specific analyte concentrations above the EPA target LOQ need to be rerun.

3.4 Percent Recovery of the Internal Standards

A group of carbon-labeled PCDD/PCDF congeners is added to every sample before the extraction. The role of the internal standard is to quantify the indigenous analyte present in the samples (isotope-dilution mass spectrometry) as well as to determine the overall method efficiency. Recoveries of the internal standards should be between 50% to 150%. If the recovery goal is not met, corrective action similar to that described above should be performed except that only those samples not meeting 50% - 150% criterion need to be reanalyzed.

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Table 1. Sample Size and 2,3,7,8-TCDD-Based Method Calibration Limits for Redacted

Lower MCL[a] (ppb)	0.0005
Upper MCL[a] (ppb)	0.4
Weight (g)	10.0
IS Spiking Level (ppb)	0.1
Final Extract Volume (µL)	20

(a) Method Calibration Limits (MCLs) = the concentration in the sample that yields an extract that has the same concentration as that of the lowest/highest calibration point.

For other congeners: Multiply the values by 1 for TCDD, 5 for PeCDD, 25 for HxCDD, 10 for TCDF, 50 for PeCDF, 40 for HxCDF, and 250 for HpCDF.

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Table 2. Composition of the Sample Fortification and Recovery Standard Solutions

Compound	Internal Standard Spiking Solution ng/mL	Matrix Spike Fortification Solution ng/mL	Recovery Standard Solution ng/mL
2,3,7,8-TCDD	--	1	--
2,3,7,8-TCDF	--	1	--
1,2,3,7,8-PeCDD	--	5	--
1,2,3,7,8-PeCDF	--	5	--
2,3,4,7,8-PeCDF	--	5	--
1,2,3,4,7,8-HxCDD	--	5	--
1,2,3,6,7,8-HxCDD	--	5	--
1,2,3,7,8,9-HxCDD	--	5	--
1,2,3,4,7,8-HxCDF	--	5	--
1,2,3,6,7,8-HxCDF	--	5	--
1,2,3,7,8,9-HxCDF	--	5	--
2,3,4,6,7,8-HxCDF	--	5	--
1,2,3,4,6,7,8-HpCDD	--	5	--
1,2,3,4,6,7,8-HpCDF	--	5	--
1,2,3,4,7,8,9-HpCDF	--	5	--
$^{13}C_{12}$-2,3,7,8-TCDD	1	--	--
$^{13}C_{12}$-2,3,7,8-TCDF	1	--	--
$^{13}C_{12}$-1,2,3,7,8-PeCDD	1	--	--
$^{13}C_{12}$-1,2,3,7,8-PeCDF	1	--	--
$^{13}C_{12}$-2,3,4,7,8-PeCDF	1	--	--
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	1	--	--
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	1	--	--
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	1	--	--
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	1	--	--
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	1	--	--
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	1	--	--
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	1	--	--
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	1	--	--
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	1	--	--
$^{13}C_{12}$-1,2,3,4-TCDD	--	--	200
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	--	--	200

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Table 3. The Fifteen 2,3,7,8-Substituted PCDD and PCDF Congeners

PCDD	PCDF
2,3,7,8-TCDD(*)	2,3,7,8-TCDF(*)
1,2,3,7,8-PeCDD(*)	1,2,3,7,8-PeCDD(*)
1,2,3,6,7,8-HxCDD(*)	2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDD	1,2,3,6,7,8-HxCDF
1,2,3,7,8,9-HxCDD(+)	1,2,3,7,8,9-HxCDF
1,2,3,4,6,7,8-HpCDD(*)	1,2,3,4,7,8-HxCDF(*)
	2,3,4,6,7,8-HxCDF
	1,2,3,4,6,7,8-HpCDF(*)
	1,2,3,4,7,8,-HpCDF

(*) The ^{13}C-labeled analog is used as an internal standard.
(+) The ^{13}C-labeled analog is used as a recovery standard.

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Table 4. **Isomers of Chlorinated Dioxins and Furans as a Function of the Number of Chlorine Atoms**

# of Chlorine Atoms	# of Dioxin Isomers	# of 2,3,7,8 Isomers	# of Furan Isomers	# of 2,3,7,8 Isomers
1	2	--	4	--
2	10	--	16	--
3	14	--	28	--
4	22	1	38	1
5	14	1	28	2
6	10	3	16	4
7	2	1	4	2
8	1	1	1	1
Total	**75**	**7**	**135**	**10**

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Table 5. Calibration Solution Concentrations

Compound	Solution Concentration (ng/mL)					
	CS0.5	CS1	CS2	CS3	CS4	CS5
Native CDDs and CDFs						
2,3,7,8-TCDD	0.25	0.5	2	10	40	200
2,3,7,8-TCDF	0.25	0.5	2	10	40	200
1,2,3,7,8-PeCDD	1.25	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	1.25	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	1.25	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	1.25	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	1.25	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	1.25	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	1.25	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	1.25	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	1.25	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	1.25	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	1.25	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	1.25	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	1.25	2.5	10	50	200	1000
Labeled CDDs and CDFs						
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100	100
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100	100

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Table 5. Calibration Solution Concentrations (Continued)

Compound	Solution Concentration (ng/mL)					
	CS0.5	CS1	CS2	CS3	CS4	CS5
Cleanup Standard						
$^{37}Cl_4$-2,3,7,8-TCDD	0.25	0.5	2	10	40	200
Recovery Standards						
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100	100

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Table 6. Ions Monitored for HRGC/HRMS Analysis of PCDDs/PCDFs*

Descriptor	Accurate[a] Mass	Ion ID	Elemental Composition	Analyte
1	303.9016	M	$C^{12}H_4{}^{35}Cl_4O$	TCDF
	305.8987	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF
	315.9419	M	$^{13}C_{12}H_4{}^{35}Cl_4O$	TCDF (S)
	317.9389	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO$	TCDF (S)
	319.8965	M	$C_{12}H_4{}^{35}Cl_4O_2$	TCDD
	321.8936	M+2	$C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD
	327.8847	M+8	$C_{12}H_4{}^{37}Cl_4O_2$	TCDD
	331.9368	M	$^{13}C_{12}H_4{}^{35}Cl_4O_2$	TCDD (S)
	333.9338	M+2	$^{13}C_{12}H_4{}^{35}Cl_3{}^{37}ClO_2$	TCDD (S)
	375.8364	M+2	$C_{12}H_4{}^{35}Cl_5{}^{37}ClO$	HxCDPE
	[292.9825]	LOCK	C_7F_{11}	PFK
2	339.8597	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF
	341.8567	M+4	$C_{12}H_3{}^{35}Cl^3{}_{37}Cl_2O$	PeCDF
	351.9000	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)
	353.8970	M+4	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO$	PeCDF (S)
	355.8546	M+2	$C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD
	357.8516	M+4	$C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD
	367.8949	M+2	$^{13}C_{12}H_3{}^{35}Cl_4{}^{37}ClO_2$	PeCDD (S)
	369.8919	M+4	$^{13}C_{12}H_3{}^{35}Cl_3{}^{37}Cl_2O_2$	PeCDD (S)
	409.7974	M+2	$C_{12}H_3{}^{35}Cl_6{}^{37}ClO$	HpCDPE
	[354.9792]	LOCK	C_9F_{13}	PFK
3	373.8208	M+2	$C^{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF
	375.8178	M+4	$C^{12}H_2{}^{35}Cl_4{}^{37}Cl_2O$	HxCDF
	383.8639	M	$^{13}C_{12}H_2{}^{35}Cl_6O$	HxCDF (S)
	385.8610	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO$	HxCDF (S)
	389.8156	M+2	$C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD
	391.8127	M+4	$C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD
	401.8559	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD (S)
	403.8529	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD (S)
	445.7555	M+4	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$	OCDPE
	[392.9760]	LOCK	C_9F_{15}	PFK

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Table 6. Ions Monitored for HRGC/HRMS Analysis of PCDDs/PCDFs (Continued)*

Descriptor	Accurate[a] Mass	Ion ID	Elemental Composition	Analyte
4	407.7818	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	409.7788	M+4	$C^{12}H^{35}Cl_5{}^{37}Cl_2O$	HpCDF
	417.8253	M	$^{13}C_{12}H^{35}Cl_7O$	HpCDF (S)
	419.8220	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO$	HpCDF
	423.7767	M+2	$C_{12}H^{35}Cl_6{}^{37}ClO_2$	HpCDD
	425.7737	M+4	$C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
	435.8169	M+2	$^{13}C_{12}H^{35}Cl_6{}^{37}ClO^2$	HpCDD (S)
	437.8140	M+4	$^{13}C_{12}H^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD (S)
	479.7165	M+4	$C_{12}H^{35}Cl_7{}^{37}Cl_2O$	NCDPE
	[430.9728]	LOCK	C_9F_{17}	PFK

(a) The following nuclidic masses were used:

H	= 1.007825	C	= 12.00000
O	= 15.994915	^{13}C	= 13.003355
^{79}Cl	= 78.91834	^{81}Cl	= 80.91629
^{35}Cl	= 34.96885	^{37}Cl	= 36.9659
^{19}F	= 18.9984		

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Table 7. Gas Chromatography/Mass Spectrometry Conditions

Column type	DB-5 or equivalent
Length (m)	60
i.d. (mm)	0.32
Film Thickness (µm)	0.25
Carrier Gas	Helium
Carrier Gas Flow (mL/min)	1
Injection Mode	On column
Injector Temperature (C)	140
Program Temperature	140°C, hold 3 min, ramp to 220°C at 20°C/min, hold for 16 min, ramp to 235°C at 5°C/min, hold for 7 min, ramp to 320°C at 5°C/min, hold 10 min
Ionization Mode	EI
Resolution	~10,000 (10% Valley definition)
Source Temperature	~280°C
GC/MS Interface Temperature	~300°C
Masses Monitored (SIM mode)	see Table 6
Cycle Time (SIM mode)	≤1 second
Acquisition Time	~19 min – 52 min

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Table 8. Theoretical Ion Abundance Ratios and Their Control Limits for PCDDs and PCDFs

Number of Chlorine Atoms	Ion Type	Theoretical Ratio	Control Limits	
			Lower	Upper
4	$\frac{M}{M+2}$	0.77	0.65	0.89
5	$\frac{M+2}{M+4}$	1.55	1.32	1.78
5	$\frac{M+2}{M+4}$	1.24	1.05	1.43
5[a]	$\frac{M}{M+2}$	0.51	0.43	0.59
7[b]	$\frac{M}{M+2}$	0.44	0.37	0.51
7	$\frac{M+2}{M+4}$	1.04	0.88	1.20
8	$\frac{M+2}{M+4}$	0.89	0.76	1.02

a Used only for ^{13}C-HxCDF (IS).
b Used only for ^{13}C-HpCDF (IS).

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SAMPLING PROTOCOL

DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN REDACTED

Study Number
AG000008

Test Substance:

Redacted (CAS # Redacted)

Prepared For:

Albaugh, Inc.
121 NE 18th Street
Ankeny, IA 50021

Battelle
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SAMPLING PROTOCOL
DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN
REDACTED

APPROVED:

Ron Collins — 3-16-00
Ron Collins
Study Director
Albaugh, Inc.
Date

James Kahnk — 3-16-00
James Kahnk
Operations Manager
Albaugh, Inc.
Date

John F Stadalsky — 3-15-2000
John Stadalsky
Quality Assurance
Blackman Uhler Chemical
Date

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SAMPLING PROTOCOL
DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN REDACTED

1.0. Principals

1.1 Sponsor: Albaugh, Inc.

121 NE 18th Street
Ankeny, IA 50021
Phone: (515) 964-9444
Fax: (515) 964-7813

Study Director - Ron Collins
Phone: (816) 238-3377
Fax: (816) 238-3938

1.2 Sampling Facility: Blackman Uhler Chemical
Division of Synalloy Corporation
Augusta Chemical Plant
1010 Glass Factory Ave.
Augusta, GA 30901

2.0 Proposed Experimental Start Date July 1, 2000

3.0 Proposed Experimental Termination Date August 1, 2000

4.0 Data Requirements

U.S. Environmental Protection Agency (EPA) Toxic Substance Control Act (TSCA); Good Laboratory Practice Standard, 40 CFR Part 792; and the Dioxin/Furan Test Rule, 40 CFR Part 766. Under these provisions, this study is defined as a physical and chemical characterization study designed to determine certain physical and chemical characteristics of the test substance redacted (see 792.135(b); (54 FR 34094)).

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5.0 Objective

The purpose of this study is to obtain samples of redacted that are typical of the material imported by Albaugh, Inc. for manufacturing. Samples of redacted manufactured by Anupam Rasayan (1661 Suthar Street, Nanpura, Surat 395001, India) will be sampled at Albaugh, Inc.'s toll manufacturing plant; Blackman Uhler Chemical (1010 Glass Factory Ave., Augusta, GA 30903).

The samples will be shipped to Battelle where the samples will be analyzed for PCDD/PCDF under the direction of Dr. Mark Bauer. Analyses will be conducted according to the "Analysis Protocol for Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Redacted". The analysis results will be used to meet regulatory requirements promulgated by the U.S. Environmental Protection Agency; Dioxin/Furan Test Rule, 40 CFR 766.

6.0 Test Substance and Test System

6.1 Test and Substance

The test substance for this study is listed below. Albaugh, Inc. will maintain a certificate of analysis and other chemical and physical characterization as required by Good Laboratory Practice Standards. Albaugh, Inc. will also maintain the stability, storage conditions, and will maintain the documentation for the synthesis and characterization of the test substances.

Common Name:	redacted
Chemical Name:	redacted
CAS Number:	redacted
Empirical Formula:	redacted
Molecular Weight:	redacted
Structure	redacted

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6.2 Test System

The test systems for this study will be redacted.

6.3 Justification for Selection of the Test System

The test system for this study is the same as the test substance upon which all analyses will be conducted. The test system chosen is the actual substance to which the Dioxin/furan Test Rule applies.

6.4 Identification of the Test System

All test system samples will be labeled with a unique sample identification code. A unique identification code will be used so that no sample numbers are repeated.

Each sample will be identified by the following code using information taken from the barrel label's batch number and drum number:

BB-MM-NNN-BB-D

Where: BB = batch number information

MM = manufacturer information in batch number

NNN = compound name information in batch number

BB = batch number information

D = randomly selected drum number

For example, "12-AR-RED-34-3" represents the 3rd drum from batch 12-AR-RED-34 of redacted manufactured by Anupam Rasayan.

7.0 Experimental Design

7.1 Background

The code of Federal Regulations, Title 40, parts 707 and 766 (40 CFR 707 and 766) presents a rule for testing whether certain specified chemical substances may be contaminated with halogenated dibenzo-p-dioxins and dibenzofurans (The Dioxin/Furan Test Rule). This protocol describes procedures for sampling

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redacted imported by Albaugh, Inc. for use in the production of their commercial product. The samples will be analyzed by Battelle for the determination of 2,3,7,8-substituted tetra- through hepta- chlorinated dibenzo-p-dioxins and dibenzofurans following an analysis protocol, which is presented as a separate document.

7.2 Normal Production Process

The redacted is manufactured by Anupam Rasayan, in India. Redacted is produced in a batch process. Benzene is chlorinated to 1,4-dichlorobenze and then nitrated to form 2,5-dichloronitrobenzene. This product is redacted to form redacted which is then converted to a redacted before it is redacted to form redacted.

The finished product is a liquid material and is packaged into closed-head, galvanized drums for shipment. Once in the drums, redacted solidifies.

7.3 Sampling Design

By means of random sampling, a minimum of seven (7) samples of redacted will be taken. Each sample will be taken from a different manufacturing batch.

7.3.1 Definition of Product Cycle

The production cycle is one batch per day. Each batch contains approximately 1250 kg of finished product. Five 250 kg drums are filled per batch.

7.3.2 Sampling Site

Sampling will take place after redacted is imported into the United States. It is not possible to control sampling and insure GLP compliance at the manufacturing plant in India. Sampling after import, at the end use site, also ensures that the samples collected are representative of the material used by Albaugh, Inc. Samples will be collected at Albaugh's toll manufacturing facility, Blackman Uhler Chemical, 1010 Glass Factory Avenue, Augusta GA 30903.

7.3.3 Selection of Samples

The redacted is imported in container load quantities. Each container holds 64 drums which represents more than 12 batches. A minimum of 7 different batches will be sampled. The drum sampled from each batch

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will be randomly selected by using a random number generator. A programmable calculator or computer program will be used to generate random numbers between 1 and 5. For example, Microsoft Excel could be programmed with the following formula:

=ROUNDUP(RAND()*5,0)

An example completed random number selection is shown in the table below.

Batch #	Drum #
1	2
2	4
3	5
4	1
5	3
6	2
7	4

7.3.4 Sampling Process

The sampling procedures are those used for regular process quality control. Personnel collecting samples will wear appropriate protective clothing and follow all appropriate safety procedures when working with redacted.

The selected drum will be placed in a steam cabinet to re-liquefy the material. The drum will be opened and stirred to insure the contents are homogeneous. A sampling probe will be inserted into the middle of the drum to withdraw at least 100 g of redacted. The sample will be split into two 50-g sub-samples by pouring approximately half of the withdrawn sample into two clean, pre-weighed, properly labeled containers. One sub-sample will be labeled as the analytical sample, and the other labeled as a retain sample.

7.3.5 Sample Storage and Shipment

Sample container caps will be sealed with tape and the samples stored at ambient temperature.

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The analytical sub-samples will be packaged for shipment to the analytical laboratory. The containers will be placed inside a secondary container (with any appropriate padding) to ensure the integrity of the other samples should a sample container break or leak during shipping. The samples will be shipped at ambient temperature using standard DOT shipping containers and following all relevant DOT shipping regulations.

The samples will be sent via an overnight carrier to:

Dr. Mark Bauer
Battelle
505 King Avenue
Columbus, OH 43201
(614) 424-3913

7.4 Documentation of Sampling

Sample data is recorded in sample labels, the Sample Data Form, the Chain-of Custody Form, and a sampling notebook.

7.4.1 Sample Labels

All sample containers will be labeled as follows:

Redacted
Analytical Sub-sample (or Retain Sub-sample)
Identification Number: _____
Sampling Date: _____
Sampling Time: _____
Weight (net): _____
Initials:
Store at room temperature

7.4.2 Sampling Data Form

A sampling data form will be used to record information about the samples. An outline of the sampling data form is as follows:

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REDACTED SAMPLE COLLECTION DATA FORM

Date	Time	Sample ID	Container Weight	Sample + Container Weight	Sample Weight	Sampler's Initials

7.4.3 Chain-of-Custody Form

A properly completed chain-of-custody form, containing a complete record of sample disposition will be used to trace the samples. The chain-of-custody form will accompany samples during all sample shipments and/or transfers of custody. An example Chain-of-Custody Form is attached in Appendix A.

7.4.4 Sampling Record Book

In addition to the forms above, a complete record of sampling events and data will be maintained in a sampling record book or notebook. Data to be entered in this notebook includes: date, time, name of sampler, sample ID numbers, product batch or other drum label information, as well as any comments pertaining to the sampling. The notebook will also include a brief description of the sampling process and equipment used. A complete record of sample disposition, including date of shipments, mode of shipment, shipping tracking numbers, and address of receiver will be maintained in the notebook. Any deviations from written procedures, as well as any corrective actions taken on equipment will be recorded.

7.5 Corrective Action

Each sample is split into two duplicate sub-samples one of which is retained for replacement if needed. In addition, samples in excess of the minimum required may be taken such that a compromised sample may be replaced.

8.0 Control of Experimental Bias

The experimental design incorporates random sampling to control for experimental bias.

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9.0 Records to be Maintained

All raw data and written records concerning the study will be part of notebooks established for the study. The study records will include, but not necessarily be limited to, the following:

1. This protocol, and any protocol amendments or deviations;

2. Test substance identification records, any characterization records supplied by the test substance manufacturer, and shipping and receipt records;

3. A full description of sampling conducted, including a description of the any equipment used;

4. Study raw data including:

   - Sample Collection Forms
   - Random number generation data

5. The final report and any amendments thereto.

10.0 Report

A final report detailing the sampling will be prepared by the Study Director. The final report will include a signed compliance statement attesting to whether the sampling was conducted in accordance with TSCA Good Laboratory Practice Standards.

The sampling report will be included, in it's entirety, as an appendix to the analytical analysis report.

11.0 Archive Statement

The study notebook(s), raw data, and other study records will be stored by Battelle, Columbus, Ohio after the approval of the final report. A copy of the final report will be archived with the study records after all approvals have been obtained.

All retained test substance will be maintained by Albaugh, Inc.

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APPENDIX A

EXAMPLE FORMS

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CHAIN-OF-CUSTODY FORM

Study Number: _____

Item Number	Sample Description	Sample I.D.
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Relinquished By: _____ Time/Date: _____ Received By: _____ Time/Date: _____

Relinquished By: _____ Time/Date: _____ Received By: _____ Time/Date: _____

Relinquished By: _____ Time/Date: _____ Received By: _____ Time/Date: _____

Relinquished By: _____ Time/Date: _____ Received By: _____ Time/Date: _____

Relinquished By: _____ Time/Date: _____ Received By: _____ Time/Date: _____

Relinquished By: _____ Time/Date: _____ Received By: _____ Time/Date: _____

Distribution: Original – Accompany Shipment
1 copy – Project File

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QUALITY ASSURANCE PROJECT PLAN FOR THE DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN Redacted

Data Requirement:

Dioxin/Furan Test Rule, 40 CFR § 766

QAPP Guidance Document:

"Guidelines for the Determination of Halogenated Dibenzo-p-dioxins and Dibenzofurans in Commercial Products, Appendix B"

Prepared For:

Albaugh, Inc
121 N.E. 18th Street
Ankeny, IA 50021

Prepared By:

Mark R. Bauer, Ph.D.

MARCH 2000

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3.0 PROJECT DESCRIPTION AND ORGANIZATION

In order to satisfy requirements for testing chemical substances that may be contaminated with halogenated dibenzo-p-dioxins and dibenzofurans as defined in Section 4 of U.S. Environmental Protection Agency (EPA) Toxic Substance Control Act (TSCA), 15 USC 2603 and 40 CFR § 766.3 and requirements for reporting under Section 8 of TSCA, 15 USC 2607, Albaugh Inc. has contracted with Battelle to perform the dioxin/furan testing following 40 CFR part 766 on redacted.

Redacted (redacted, CAS No. redacted) imported by Albaugh, Inc. will be sampled at their toll manufacturing plant where redacted is used in the production of Albaugh's commercial product. The samples will be sent to Battelle to be analyzed for polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF).

Sampling will be conducted in accordance with the sampling protocol entitled "Sampling Protocol for the Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Redacted". Redacted manufactured by Anupam Rasayan, 1661, Suthar Street, Nanpura, Surat – 395001, India will be sampled for analysis. Randomly selected barrels from seven different batches of redacted will be sampled at Albaugh's toll manufacturing plant; Blackman Uhler Chemical, 1010 Glass Factory Avenue, Augusta, GA 30903.

The samples will be sent to Battelle, 505 King Ave., Columbus, OH 43201 for analysis. The samples will be analyzed in accordance with the analytical protocol entitled "Analytical Protocol for the Determination of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Redacted". The analytical method outlined in the analysis protocol is designed to meet regulatory requirements promulgated by the U.S. Environmental Protection Agency, Dioxin/Furan Test Rule, 40 CFR 766.

PCDD/PCDF analytes will be quantitated at or below the following target levels of quantitation (LOQs):

Chlorinated Dioxins	LOQs
2,3,7,8-TetraCDD	0.1 ppb*
1,2,3,7,8-PentaCDD	0.5 ppb
1,2,3,4,7,8-HexaCDD	2.5 ppb
1,2,3,6,7,8-HexaCDD	2.5 ppb
1,2,3,7,8,9-HexaCDD	2.5 ppm
1,2,3,4,6,7,8-HeptaCDD	100 ppb

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Chlorinated Furans	LOQs
2,3,7,8-TetraCDF	1 ppb
1,2,3,7,8-PentaCDF	5 ppb
1,2,3,7,8-PentaCDF	5 ppb
1,2,3,4,7,8-HexaCDF	25 ppb
1,2,3,4,7,8-HexaCDF	25 ppb
1,2,3,7,8,9-HexaCDF	25 ppb
2,3,4,6,7,8-HexaCDF	25 ppb
1,2,3,4,6,7,8-HeptaCDF	1000 ppb
1,2,3,4,7,8,9-HeptaCDF	1000 ppb

* ppb = part per billion or ng/g

Seven redacted samples will be analyzed. Quality control steps will be taken to ensure the quality of the data. One of the seven product samples will be randomly selected and analyzed in duplicate. One of the seven product samples will be randomly selected and analyzed both spiked and unspiked with target PCDD/PCDF analytes. These samples will demonstrate the test rule requirements for method sensitivity and precision can be met. A laboratory method blank will also be analyzed with the seven product samples to demonstrate the absence of PCDD/PCDF or non-PCDD/PCDF interferences.

The seven test samples and the quality assurance samples (duplicate, matrix spike, and laboratory method blank) will constitute the sample set. All ten samples will be processed together for a valid demonstration of the quality of the test data.

Each sample will be spiked with isotopically labeled PCDD/PCDF used as internal standards. Possible PCDD/PCDF will be extracted and the extracts cleaned up using several chromatography columns. PCDD/PCDF determination will be performed using Gas Chromatography/Mass Spectrometry in the Selected Ion Monitoring mode (SIM-GC/MS).

The analytical data will be collected, processed, and reported as described in this document.

4.0 MANAGEMENT AND PERSONNEL QUALIFICATIONS

Ron Collins, the Study Director for the sampling protocol, is the Product Development Manager at Albaugh's formulation and packaging facility. Ron Collins reports to Jim Kahnk, Operations Manager for Albaugh. John Stadalsky, the Quality Assurance Officer for sampling, is the Chief Chemist at Blackman Uhler manufacturing plant and

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manages the plant's Quality Control Department. The sampling will be conducted by a chemist in the Quality Control Department. John Stadalsky reports to Jeter Starnes, Blackman Uhler's Vice President of Research and Development

Mark Bauer, the Study Director for the analytical protocol, is the Technical Leader for Product Characterization in Battelle's AgriFood Market Sector. Mark reports to Cora Steginsky, AgriFood Market Sector Vice President and General Manager. The analyses will be conducted by staff in Battelle's Atmospheric Sciences and Applied Technology Department. The samples will be prepared for analyses by Mark Misita and Andrew Savage. The prepared samples will be analyzed by Joe Tabor. All the analytical work will be conducted in a laboratory dedicated to dioxin analyses. Chuck Lawrie, the Quality Assurance Officer, serves as the Quality Assurance Manager for both the AgriFood and the Atmospheric Sciences and Applied Technology groups. Mark Misita, Andrew Savage, Joe Tabor, and Charles Lawrie report to Karen Riggs, Deputy Manager for the Atmospheric Sciences and Applied Technology Department.

A reporting structure block diagram is shown in Figure 1. Curriculum vitae for the sampling Study Director and Quality Assurance Officer are provided in Appendix A. Curriculum vitae for the analysis Study Director and Quality Assurance Officer are also provided in Appendix A.

5.0 ANALYSIS FACILITIES, EQUIPMENT, AND SERVICES

5.1 Facilities

Any work related to PCDD/PCDF determination including the preparation, handling, and storage of all samples and standards is conducted within a laboratory dedicated to dioxin/furan analysis. The dioxin/furan laboratory has extensive experience in the field of trace environmental analysis. The laboratory has been determining dioxins and related compounds since the early 1970s. The laboratory has conducted many studies in response to the EPA regulations for testing certain chemicals for dioxins and furans under TSCA (40 CFR 766, 52 FR 21437, June 5, 1987). All submitted studies have been considered as acceptable by EPA.

5.2 Inspections and Maintenance

Written standard operating procedures regarding methods, materials, and schedules to be used for the routine inspection, cleaning, maintenance, testing, calibration, and standardization of equipment are maintained in the laboratory. Written records are maintained of all operations.

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Examples of routine operations:

certification of balances: every 12 months;

calibration of thermometers: every 12 months;

inspection of GC/MS instrument: daily, maintenance every 12 months or on instrument demand;

qualification of GC/MS data system: every 12 months or on software or hardware upgrades

inspection of glassware cleanliness: a blank is run through the entire analytical procedure with every set of samples.

5.3 Calibration Procedures

The performance of the GC/MS system is checked following the procedures detailed in laboratory standard operating procedures. The instrument calibration method is similar to that proscribed by EPA Method 1613.

On a daily basis, aliquots of the calibration solutions containing native and isotopically labeled PCDD/PCDF compounds are analyzed to demonstrate adequate sensitivity, response factor reproducibility (±20% for native analytes and ±35% for ^{13}C-labeled compounds), mass range calibration, and column performance. If the required criteria are not met, corrective actions, such as instrument re-tuning, re-analysis of the calibration solution, or generation of a new initial calibration curve, must be taken before samples are analyzed.

5.4 Calibration Materials

Standard compounds used in this study are obtained from Cambridge Isotopes Laboratories, Inc. (CIL), Woburn, MA, USA.

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6.0 DATA GENERATION

6.1 Sample Collection

The sample collection is described in the GLP sampling protocol. The protocol details how the samples will be randomly selected, generated, and handled. Sampling methods are based those presented in "Guidelines for the Determination of Polyhalogenated Dibenzo-p-dioxins Dibenzofurans in Commercial Products, Appendix C". The protocol also includes procedures for shipping the samples to the analytical laboratory.

6.2 Sample Custody

A chain-of-custody (COC) document will be generated by the sampling team. The COC will travel with samples and will document who has control of the samples and the condition of the samples before and after all transfers of control. The samples arriving in the analytical laboratory are checked for being properly labeled and packed. Upon receipt, the samples are recorded into the sample receipt logbook and all accompanying shipping records are saved in the study file. A unique laboratory sample-identification number is assigned to each sample and consistently recorded by the analyst on every container used during the course of the sample preparation and analysis.

After the analyses have been completed, the samples will be returned to Albaugh, Inc. for archive.

6.3 Laboratory Analysis Procedures

The sample analysis is described in the GLP analysis protocol. The analysis protocol details how the samples will be extracted, cleaned up, and analyzed. Analytical test methods are based those presented in "Guidelines for the Determination of Polyhalogenated Dibenzo-p-dioxins Dibenzofurans in Commercial Products", EPA Method 8290, and EPA Method 1613. The protocol includes a description of instrumental calibration procedures, how analytes are identified, equations used to calculate results, and Quality Assurance Requirements.

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6.4 Quality Control Checks

Quality Control checks are performed to monitor glassware, instrument, and analysis performance. Analysis performance data are obtained by use of blank samples, spiked samples, and repeated measurements. Method blanks, duplicates, and spiked samples are included in every set of samples. All quality-check data are included with the study results.

6.5 Performance and System Audits

During testing for submission to EPA, the laboratory adheres to Good Laboratory Practices (GLPs). Qualified independent Quality Assurance Specialists are responsible for monitoring each study to assure conformance to regulations, protocols, and appropriate SOPs. Records pertaining to the study will be archived by the testing facility Quality Assurance Unit for a period of at least 10 years following the date on which the results are submitted to EPA. This includes, but is not limited to, all raw data, protocols, reports, and other documentation.

7.0 DATA PROCESSING

7.1 Collection of GC/MS Data

The GC/MS instrument is interfaced to a validated computer system that allows continuous acquisition and storage of the analytical data obtained throughout the duration of the chromatographic programs.

7.2 Data Reduction and Verification

Data reduction is accomplished using a validated software program designed for dioxin/furan analysis by the mass spectrometer manufacturer. The criteria applied for PCDD/PCDF identification and quantitation methods used are the same as those specified by the analysis protocol. Details of quantitative calibration procedures and the relevant equations for performing mathematical calculations are given in the analytical protocol. Any additional statistical calculations required, specific to this study, will be accomplished with a spreadsheet program.

Data review procedures include computerized and manual checks. The computer data system flags analysis results outside preset acceptance criteria. Calculations

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will be checked from the raw data to the final value reported. Prior to review by the Quality Assurance Unit, the data will be manually reviewed by a second analyst who will verify that data reduction has been performed correctly and that the analytical results correspond to the data acquired and processed.

7.3 Storage

SIM-GC/MS raw data are stored on magnetic hard disks; hard copies of processed chromatograms and derived data are produced and saved separately.

8.0 DATA QUALITY ASSURANCE

Prior to sample cleanup, internal standards are added to every sample in order to provide an indication of the method performance with every sample analysis. Quality control steps are taken to ensure the quality of the data. These include the analysis of a laboratory method blank, a sample analyzed in duplicate, and a sample run spiked with native PCDD/PCDF compounds along with the product samples.

8.1 Laboratory Method Blank

Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts or elevated baselines that interfere with interpretation of the analytical data. Analysis of the laboratory method blank demonstrates the absence of interference from these sources. To that effect, all steps detailed in the analytical procedure using all reagents, standards, equipment, apparatus, glassware, and solvents that are used for a sample analysis (omitting the product sample) are performed. The laboratory method blank contains the same amount of $^{13}C_{12}$-labeled internal standards that is added to each sample before extraction.

An acceptable method blank exhibits no positive response (i.e., no response for specific analytes that exceeds the target analytical method LOQ). If the method blank that is extracted along with a batch of samples is contaminated by PCDD/PCDF compounds, all positive samples from that batch must be rerun if they are positive for the same analyte(s) for which the blank is positive.

8.2 Precision

The test rule requirement for duplicate data is fulfilled by analysis of the duplicate samples which both contain the isotopically labeled PCDD/PCDF compounds used as internal standards. The duplicates must agree within 20%.

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If the relative percent difference between recoveries is greater than 20 for anyone of the internal standards, the laboratory will attempt to identify the cause of the problem and repeat the analysis of the duplicates.

8.3 Accuracy

Labeled PCDD/PCDF compounds are added to very sample before the extraction to quantify the indigenous analyte present in the samples as well as to determine the overall method efficiency. For each set of samples, one portion of redacted sample is additionally spiked with a mixture of native PCDDs and PCDFs.

The recovery of the native PCDD/PCDF homologs are calculated by comparing the spiked and unspiked sample runs. Recovery of the native PCDDs and PCDFs must be in the range of 50 to 150%.

Satisfactory recoveries of the internal standards from the laboratory method blank must be verified. If interferences are encountered that cause recoveries of the internal standards in the blank to be greater than 150% or if the recoveries are less than 50%, the cause should be identified and appropriate procedural changes performed.

For each sample, the recovery of the internal standards must be in the range 50 to 150%.

The samples not meeting the 50 to 150% criterion need to be reanalyzed.

8.4 Completeness

100% of the samples taken are analyzed. The objective of completeness is 100% calculated as 100 x number of values recovered / number of recoverable values.

8.5 Comparability

On various occasions, the dioxin/furan laboratory has successfully participated and participates in national or international multilaboratory dioxin studies including the study that verified the concentrations of the analytical standards sold by Cambridge Isotope Laboratories.

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9.0 CORRECTIVE ACTION

Corrective actions are measures taken to rectify conditions adverse to quality and, where possible, prevent their reoccurrence. During the analyses, any corrective actions taken, not described in the protocols or in the Quality Assurance Project Plan, will be explained in the study records. The occurrence of the problem, the corrective action employed, and verification that the problem has been eliminated will be included in the explanation.

10.0 DOCUMENTATION AND REPORTING

This study will be conducted in compliance with TSCA Good Laboratory Practice Standards (40 CFR 792). All records and data generated during the conduct of the study will be generated in accordance with this standard.

The final report will be prepared following the guidelines set forth in "Guidelines for Reporting Test Results of HDD and HDF Determinations in commercial products (40 CFR parts 707 and 766)". The report will include all required GLP elements set forth in 40 CFR 792.185. The report will include appendices containing at least:

- A final sampling report, prepared by the sampling Study Director, containing a GLP compliance statement, and all sampling records;
- Sample chain-of-custody records
- Standard preparation records
- Sample extraction and clean up records
- All GC/MS data for instrument calibrations and sample analyses

The final report is audited by the Quality Assurance Unit and submitted to sponsors.

11.0 REFERENCES

1) "Guidelines for the Determination of Halogenated Dibenzo-p-dioxins and Dibenzofurans in Commercial Products, Appendix B: Quality Assurance Project Plan for Measurement of Halogenated Dibenzo-p-dioxins (HDDs) and Dibenzofurans (HDFs)", U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, EPA 560/5-87/007, September 1987.

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2) "Method 1613:Tetra-through Octa-chlorinated dioxins and Furans by Isotope dilution HRGC/HRMS", U.S. Environmental Protection Agency, Office of Water Engineering and Analysis Division, EPA 821-B-94-005, October 1994, Revision B

3) "Method 8290: Analytical Procedures and Quality Assurance for Multimedia Analysis of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans by High Resolution Gas Chromatography/High Resolution Mass Spectrometry", U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, June 1987.

4) "Guidelines for Reporting Test Results of HDD and HDF Determinations in Commercial Products (40 CFR 707 and 766)", U.S. Environmental Protection Agency, Office of Pesticides and Toxic Substances, June 22, 1990

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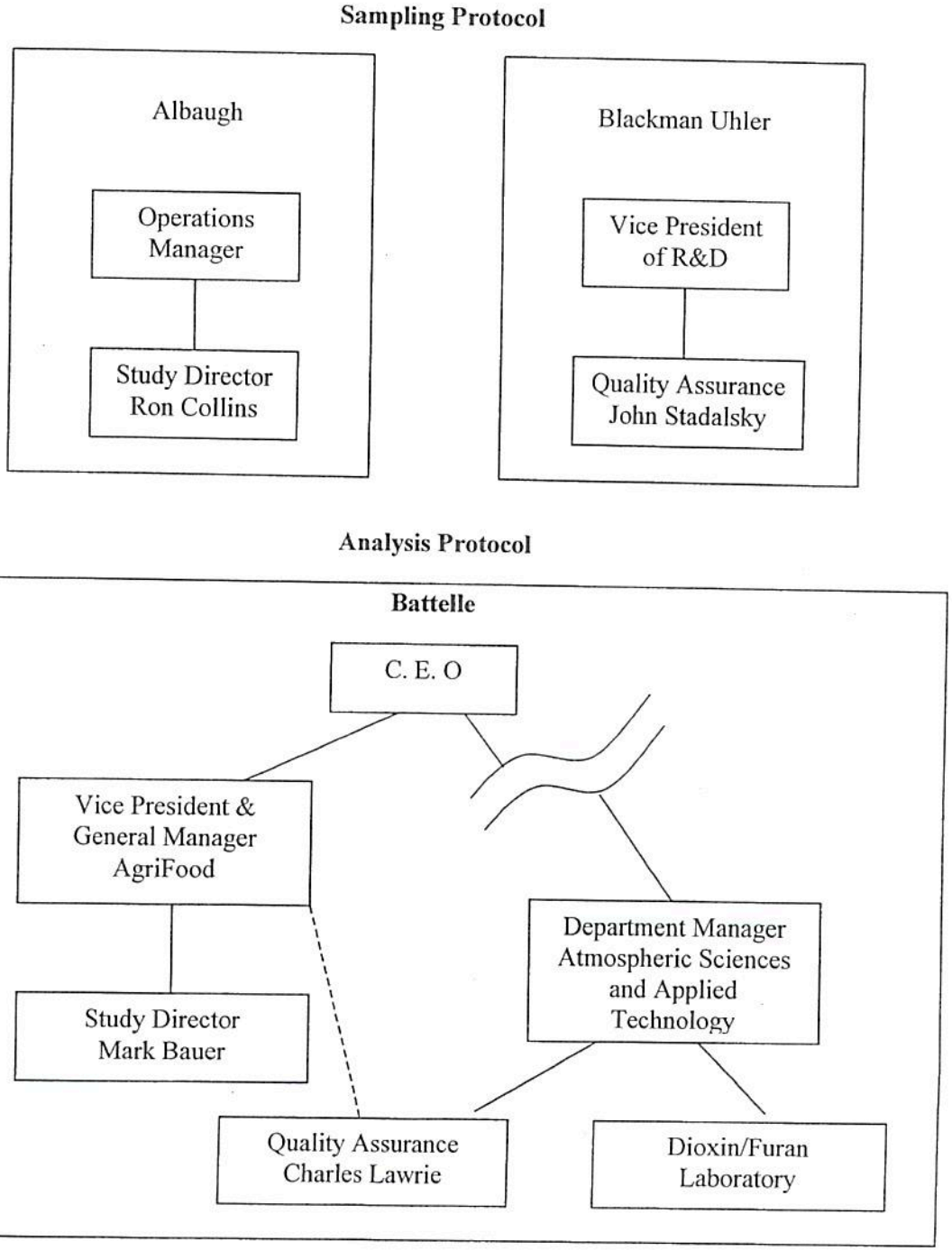
FIGURE 1. REPORTING STRUCTURE BLOCK DIAGRAM



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APPENDIX A

CURRICULUM VITAES

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Ron Collins
Albaugh, Inc.
Plant Manager, St. Joseph Facility

EDUCATION

Northwest Missouri State University — Maryville, MO
Bachelor of Science: Chemistry — May, 1969

EXPERIENCE

Albaugh, Inc. St. Joseph, MO — Jan. 1992 – Present
Title: Plant Manager
Responsible for all operational areas of this agricultural chemical manufacturing and formulating plant. Includes direct managerial responsibility for production, shipping & receiving, maintenance & engineering, order processing, quality control, and regulatory compliance.

Wilcox Electric Kansas City, MO — Aug. 1990 – Dec. 1991
Business: Manufacturer of Aviation Guidance Systems
Title: Regulatory Affairs Specialist
Responsible for ensuring plant compliance with state and federal environmental and hazardous materials regulations. This included hazardous waste management, right-to-know and RCRA training, environmental health and safety oversight, and hazardous chemicals inventory control.

Agrolinz Inc. St. Joseph, MO — 1983 – 1990

Business: Manufacture and Formulation of Agricultural Chemicals

Title: Laboratory Manager
Responsible for quality control, formulation & process development, and environmental regulatory compliance.

Rhone-Poulenc Inc. St. Joseph, MO — 1974 – 1983
Business: Formulation of Agricultural Chemicals
Title: Laboratory Supervisor
Supervised the quality control laboratory. Responsible for ensuring raw materials and finished products were in compliance with established specifications.

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Ron Collins (Continued)

EXPERIENCE (CONTINUED)

The Blueside Company St. Joseph, MO 1970 – 1974
Business: Leather Tanning
Title: Chemist
Performed quality control tests on raw materials and finished products. Purchased raw materials and maintained inventory.

PROFESSIONAL MEMBERSHIPS

American Chemical Society
Board of Directors South St. Joseph Industrial Sewer District

REFERENCES

John Faris
Harcross, Inc.
5200 Speaker Rd.
Kansas City, KS 66106
(913)321-3131

Joe Brennan
Terra International
1000 Terra Road
Blytheville, AR 72316
(501)763-2022

Terry Heath
Witco Corp.
3230 Brookfield
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(618)692-6488

Alice Walker
Alice Walker Consultants
785 Country Club Drive
Senatobia, MS 38668
(601)562-5995

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John F. Stadalsky
Blackman Uhler Chemical
Product Development Manager

EDUCATION

Clemson University — Clemson, SC
Bachelor of Science: Chemistry — June, 1975

EXPERIENCE

Blackman Uhler Chemical, Spartanburg, SC — 1980 – Present
Title: Product Development Manager
Coordinate toll and customer manufacturing projects from lab to production. Facilitate transfer of technical information and projects between the Spartanburg location and assist with the Quality Control management at the Augusta location.

Title: Research and Development Chemist — 1987-1989
Coordinated the production of water treatment chemicals, ran development program for water treatment chemicals. Assisted in the development of Textile printing chemicals and dye intermediates.

Title: Research and Development Chemist — 1980-1987
Developed and coordinated dye intermediate production. Developed and scaled-up nitration processes. Established use of online information retrieval and development projects.

Unisphere Chemical, Spartanburg, SC — 1978-1980
Title: VP – Director of Development
Responsible for R&D, QC, and helped manage production.

Milliken Chemicals, Spartanburg, SC — 1975-1978
Title: Senior Development Chemist
Developed and scaled-up ethoxylated and propoxylated products. Designed and oversaw construction of new ethoxylation and research laboratories at the Milliken Research Center. Developed dyeing and finishing auxiliaries.

Title: Development Chemist — 1970-1975
Developed and scaled-up ethoxylated intermediates, fiber finish components and dyeing auxiliaries. Developed line of printing ink dispersants.

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John F. Stadalsky (Continued)

PROFESSIONAL MEMBERSHIPS

American Chemical Society – Western Carolina Section

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Mark R. Bauer, Ph.D.
Battelle Memorial Institute
Senior Research Scientist

EDUCATION

Denison University Bachelor of Science: Chemistry	Granville, OH 1980
Michigan State University Master of Science: Analytical Chemistry	East Lansing, MI 1983
Michigan State University Ph.D.: Analytical Chemistry	East Lansing, MI 1986

EXPERIENCE

Battelle Memorial Institute, Columbus, OH 1996–Present
Title: Senior Research Scientist

Dr. Bauer is a Senior Research Scientist in Battelle's AgriFoods Market Sector (formerly Agrochemical Product Development). He has led studies involving protocol preparation, field sampling, sample transmittal, sample receipt, sample processing, analytical methods development, sample preparation and analysis, data analysis and evaluation, quality control/quality assurance, and report preparation. In addition, as a Technical Leader within the group, he has been responsible for the technical quality of studies conducted by other researchers.

Dr. Bauer has developed methods for isomer-specific determination of polychlorinated and polybrominated dibenzo-p-dioxins and dibenzofurans in a variety of matrices, including bulk chemicals, flame retardants, resins, and extrusion fumes. The results of some of this research were published in the Journal of Fire Sciences and Bull. Soc. Chim. Belges.

Dr. Bauer's studies have encompassed a variety of analytes, metabolites, and matrices. The analytes have covered various pesticide classes, including herbicides, insecticides, and fungicides. The matrices have included a wide variety of crops, soils, sediments, and waters. He has also conducted studies on a variety of animal tissues and organs. He identified pesticide metabolites using a variety of MS techniques, including EI and CI GC/MS, MS/MS, GC/MS accurate mass, and FAB. He also applied these methods to determine the chemical structures of newly synthesized materials or recently isolated chemical and biological metabolites.

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Mark R. Bauer, Ph.D. (Continued)

EXPERIENCE (CONTINUED)

In addition to his study accomplishments, Dr. Bauer has developed new instrumental analysis techniques, including a method for the ultra-trace determination of pyridostigmine bromide in plasma by packed capillary liquid chromatography/continuous flow FAB MS and a method for determining volatile organics in water using a hollow-fiber membrane MS interface. His study results were described in Analytical Chemistry.

Title: Technical Leader 1994-Present
Dr. Bauer also serves as a Technical Leader for Product Characterization, where he is responsible for project leadership and for the technical quality of chemical characterization programs which utilize a wide variety of analytical instrumental techniques to determine physical properties and/or identify and quantitate trace impurities.

Title: Principal Research Scientist 1993-1996

Title: Research Scientist 1987-1993

Michigan State University, East Lansing, MI 1980-1986
Title: Graduate Assistant

Denison University, Granville, OH 1978-1980
Title: Teaching Assistant

PROFESSIONAL MEMBERSHIPS

American Society for Mass Spectrometry
American Chemical Society

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Charles D. Lawrie
Battelle Memorial Institute
Supervisor, Quality Assurance

EDUCATION

University of Illinois at Chicago — Chicago, IL
Bachelor of Science: Biological Sciences — 1987

University of Maryland — College Park, MD
Master of Science: Marine, Estuarine, and Environmental Sciences) — 1990

EXPERIENCE

Battelle Memorial Institute, Columbus, OH — 1998–Present
Title: Supervisor, Quality Assurance
Responsible for all quality assurance matters in the Agrochemical Product Development (APD) department. Primary duties to ensure departmental compliance with 40 CFR 160 as they apply to regulated studies. Review studies to ensure compliance with current EPA regulations. Administrate APD department's training program. Assist FDA QAU in periods of high work throughput.

Nestlé Quality Assurance Laboratory — 1996-1998
Title: Vitamin Chemistry
Responsible for supervising chemical analyses and implementation of additional study teams operating under FDA guidelines. This work specializes in vitamin assays, analysis and reporting of chemical data, methods development, internal and factory laboratory audits, SOP generation, Good Laboratory Practices, Quality Assurance and Quality Control of laboratory data.

Covance Laboratories (Corning Hazleton WI) — 1991-1995
Title: Study Director
Responsible for project supervision of studies of agricultural chemicals for EPA registration under 40 CFR Part 158, Subdivision N, Sections 161-163. These studies elucidate the fate of xenobiotics under various environmental conditions. This work specializes in: pesticide product registration, degradation and transport pathways, analyses and reporting of chemical data, study and workplan design, Good Laboratory Practices (GLP), Quality Assurance and Quality Control of laboratory data.

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Charles D. Lawrie (Continued)

EXPERIENCE (CONTINUED)

United States Department of Agriculture 1990-1991
Title: Agricultural and Food Chemist
Responsible for methods development for the extraction, purification and characterization of organic compounds found in various food commodities. This work specialized in advanced analytical instrumentation using FDA and EPA Good Laboratory Practices (21 CFR Part 58 and 40 CFR 160, respectively).

PROFESSIONAL MEMBERSHIPS

American Chemical Society, Agrochemical Division
Society of Quality Assurance

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